What lessons can be learned about asthma phenotypes in children from cohort studies?

Jocelyne Just, Flore Amat, P Saint-Pierre, R Gouvis-Echraghi, N Guillemot, T Guiddir, R Couderc, Isabella Annesi-Maesano

What lessons can be learned about asthma phenotypes in children from cohort studies?

J Just[1,2], F Amat[1,2], P Saint Pierre[3], R Gouvis-Echraghi[1], N Guillemot[1,2], T Guiddir[1,2], R Couderc[4], I Annesi Maesano[2]

[1]Allergology department, Centre de l'Asthme et des Allergies. Hôpital d'Enfants Armand-Trousseau (APHP) –

[2]Sorbonne Universités, UPMC Univ Paris 06, UMR_S 1136, Institut Pierre Louis d'Epidémiologie et de Santé Publique, Equipe EPAR, F-75013, PARIS 75571 Cedex

[3]Laboratoire de statistiques théoriques et appliquées, Université Pierre et Marie Curie - Paris 06, 4 place Jussieu, 75252 PARIS cedex 05, France.

[4]Service de biochimie et biologie moléculaire, Hôpital d'Enfants Armand-Trousseau - 26, Avenue du Dr. Arnold Netter, 75571 PARIS Cedex 12 – UPMC Univ Paris 06. France.

Word (4780 /4500)

Ref (43/75)

Address for correspondence:

Pr. Jocelyne JUST

Centre de l'Asthme et des Allergies. Hôpital d'Enfants Armand-Trousseau (APHP) - 26, Avenue du Dr. Arnold Netter, 75571 PARIS Cedex 12. France.

Tel. +33 1 44 73 63 17

24 Fax: +33 1 44 73 66 35

25 E-mail: jocelyne.just@trs.aphp.fr

26

27

Abstract

"Phenotyping" asthma by multivariate analyses and more recently by unsupervised analysis has been performed in children cohorts. We describe the key findings that have emerged from these cohorts. In very young children 3 wheeze phenotypes seem to exist: the mild episodic viral wheeze phenotype, thesevere atopic wheeze and more inconstantly encountered the severe nonatopic wheeze. Early onset of allergy in asthma (more frequently encountered in boys) is associated with poor prognosis unlike the severe non-atopic wheeze phenotype with a female predominance. The prognosis of the severe nonatopic wheeze depends on time of onset (early or late) of allergic expression. At school age**, t**he risk of severe asthmatic exacerbations is associated with eosinophil predominant inflammation frequently related to allergic asthma, whereas neutrophil inflammation is associated with moderate-to-severe asthma with poorer lung function. Nevertheless, Allergic asthma is also a heterogeneous disease with severe allergic phenotype strongly associated with with atopic dermatitis and very high eosinophil-driven inflammatory markers. Further studies are required to find non-invasive biological markers to better define wheezing phenotypes in very young children associated with an elevated risk of developing severe asthma with a view to personalizing treatment.

Introduction

Asthma is the most common disease during childhood. It affects 200–300 million people worldwide, and its prevalence has increased over the past few decades[1].. It is a heterogeneous disease in terms of triggers, severity, inflammation and age of onset[2]. To date, asthma classification has mainly been related to asthma severity: it is generally accepted that phenotypes related to asthma severity follow a track during childhood and even during the whole of life[3]. This observation supports the notion that phenotypes defined by asthma severity remain stable.

Asthma severity is also an important parameter because the major healthcare burden of this disease is related to severe asthma. Nevertheless, severe asthma includes multiple features and it is important to distinguish between the different severe phenotypes to determine targeted and effective treatment[4].

Anti-inflammatory treatment is the pedestal of asthma treatment but there is ongoing debate about whether children, especially preschool children, should be treated with continuous or intermittent inhaled corticosteroid treatment. On the other hand, uncontrolled asthma despite high levels of controlled medication should be treated with an additional treatment such as an anti-IgE[5] or various anti-cytokine treatments.

There is currently growing evidence that personalized medicine based on phenotypes and endophenotypes (characterized by physiopathological pathways) is important in the management of all chronic diseases[6] For all these reasons, and especially to contribute to decreasing the burden of the disease, phenotypes deserve to be described in asthma.

Against this background of "phenotyping" asthma, multivariate analyses and, more recently, unsupervised analyses have been performed in birth cohorts [789101112131415]. In the same manner, phenotyping analyses have also been performed in cohorts of

children comprising moderate to severe asthma phenotypes[1617181920212223]. In relation to these data, it appears that age of onset, hyperresponsiveness, asthma control under treatment and multiple sensitizations are important parameters to define phenotypes of asthma during childhood.

In this article we highlight the main results, which have emerged from these cohorts in preschool then in school age wheezers

In very young children, severe asthma phenotypes exist and are related to gender (Table n°1) The Pollution and Asthma Risk an Infant Study (PARIS), a birth cohort composed of 3500 full-term newborns recruited in five public Parisian maternity hospitals in Paris and its close suburbs, described wheezer phenotypes[24]: severe atopic wheezers and non-atopic severe wheezers as well as a mild nonatopic wheezer phenotype. Trousseau Asthma Program (TAP) cohorts are independent cohorts of several hundred consecutive children explored in a cross-sectional and prospective manner by unsupervised analysis. In a TAP cohort study of 551 early wheezers with an average age of 18 months described similar asthma phenotypes by cluster analysis: "*Mild Episodic Viral Wheeze" (EVW)* and "*Atopic Multiple-Trigger*" (*AMT*) and "*Non-atopic uncontrolled-wheeze*" (*NAUW*)[14] (Fig 1). These phenotypes were validated by another independent TAP cohort[15] which also applied a new approach including exhaled nitric oxide (FeNO) determinants together with severity and atopy by cluster analysis. Furthermore

EVW consisted of children with wheeze related to cold only, mild disease. These findings are consistent with The Tucson Children's Respiratory Study. This birth cohort is a long-term, longitudinal, prospective study of the risk factors for acute lower respiratory tract illnesses in early childhood and for chronic obstructive airways disease in later life. It includes 1,246 newborns enrolled between May 1980 and January 1984[25]. For the first time these authors described early transient wheeze with rare episodes of wheeze apart from colds in infancy compared to persistent wheezers[26]. In the same manner, Spycher *et al* [27]. in two independent cohorts (nested samples of total population of 1,650 white children recruited in 1990 at the age of 0–5 years in Leicestershire, UK) showed a phenotype with mild, virus-triggered symptoms wheeze. The *AMT* phenotype, includes more children with allergic diseases such as

eczema, allergic rhinitis (AR) food allergy and more specific IgE positivity and a higher proportion of elevated values for total IgE. This phenotype is associated with severe wheezing disease, defined by a greater percentage of multiple trigger wheezes (*MTW*). The *AMT* phenotype is comparable to phenotypes identified by using other approaches. For example, in the Avon Longitudinal Study of Parents and Children (ALSPAC)[6], a birth cohort in which the children of 14 541 pregnancies were recruited antenatally between 1990–92, two phenotypes characterized by early and intermediate-onset of wheeze persisting during childhood were characterized by allergy features (with skin prick test positivity) and severity of the disease (with bronchial hyperesponsiveness and reduced lung function)[28]. This description was also confirmed across two independent cohorts of children previously described by Spycher *et al* [21]. The TAP cohort found that more boys fell into the *AMT* group. This might be related to the well-known association between male gender and allergic diseases[2930]. In a British birth cohort, The National Asthma Campaign Manchester Asthma and Allergy Study (MAAS cohort) parents were screened at 'booking' antenatal visits between October 1995 and July 1997. A total of 3,618 mothers and 2,172 fathers underwent skin-prick testing and newborns were allocated to allergic risk groups according to parental atopic status. The main result of this cohort was the impact of early onset allergy on asthma prognosis. Effectively, Belgrave *et al.*[31] showed that children with frequent asthma exacerbations and multiple early atopy are at risk of a progressive loss of lung function from 3 to 11 years and this effect is also more marked in boys.

NAUW was characterized mainly by moderate to severe disease and uncontrolled wheeze despite high doses of ICS, a higher proportion of parental asthma and higher ferritine values. This phenotype has been less described by other teams. However,

Spycher *et al.*[21], showed an intermediate phenotype between the atopic persistent and the viral wheeze phenotype in terms of prognosis in preadolescence.

Moreover, in the TAP cohort the *NAUW* phenotype was only encountered in girls. Isozaki *et al.*[32] demonstrated a gender difference in phenotypes with atopic wheezing more frequently encountered in boys and non-allergic wheezing resistant to treatment more prevalent in girls. This phenotype has been known for a long time in adults and is described as 'Intrinsic asthma' with a female predominance though the aetiology is still not understood[33].

In the TAP cohort, with only two parameters related to atopy and asthma severity (i.e. total IgE and asthma severity), 90% of boys and 83% of girls were assigned to the appropriate cluster (Fig 2). This result underlines the impact of atopy but also of the "intrinsic" aetiology in disease severity, with a higher percentage of boys in severe atopic phenotypes and girls in severe non-atopic phenotypes.

Stability of asthma phenotypes in very young children during preschool age depends on allergic expression (Table n°1)

Several rules have been developed to predict whether preschool children will have asthma at school age such as the stringent and loose forms of the Asthma Predictive Index (API) in the Tucson Cohort [34]. The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) is a birth cohort study initiated in 1996 which enrolled children born to allergic mothers in a double-blind placebo-controlled trial to evaluate the use of mite-impermeable mattress and pillow covers[35]. In this cohort, the API scores were externally validated with predictions comparable to the original Tucson study. However, these prediction rules are difficult to apply in clinical practice because of an overall low positive predictive value[36].

Another approach of the TAP cohort was to investigate if prospectively defined phenotypes could have a different course during childhood and thus improve prediction of the course of asthma (Fig 3)[17]. This study revealed a good prognosis for children classified as having the *EVW* phenotype: at 5 years old 69% were still in the *EVW* group or were asymptomatic. This finding is in accordance with many other studies which demonstrate that recurrent viral induced wheeze has a good prognosis with a low risk of asthma[37] or mild asthma. Spycher *et al*[21] in particular noted that despite mild symptoms in early life, children with transient wheeze were more likely than controls to continue to have current wheeze and use bronchodilators in preadolescence. This finding underlines the fact that remission

can occur in this phenotype, a fact which is important for the clinician in the management of early wheezing.

The prognosis of the initial severe phenotypes (*AMT* and *NAUW*) is worse than for *EVW*. This finding is in accordance with the notion of tracking asthma severity during childhood[38]. None of the children in the *AMT* phenotype became asymptomatic at 5 years and more than half of *NAUW* children were still severe at 5 years (Fig 3). This poor prognosis of allergic asthma with early onset has already been described in numerous prospective birth cohorts[203940]. The MAAS [4142] demonstrated that atopy, and especially early onset of multiple sensitizations, increases the risk of persistence of asthma with severe exacerbations during childhood. In contrast, only Spycher *et al.*[21] found a third wheeze phenotype in both their cohorts – identified as intermediate between the atopic persistent and the viral wheeze phenotype – associated with a poor prognosis in preadolescence similarly to the TAP findings for the *NAUW* group. Possibly, this phenotype is associated with persistent wheezing during preschool and school age in relation with late-onset atopy.

More globally, the lesson to retain about prognosis of the early wheezer is that changes observed in each initial phenotype in preschool aged children depend not only on the expression of allergy but also on the time of onset (early or late). Finally, although none of the phenotypes identified in the TAP corresponded to the GINA definition of asthma severity, it is worth noting that their most recent guidelines[43] have approved a "phenotyping approach" in asthma management by referencing the TAP article[17].

Asthma severity at school age depends on inflammatory cellular type (Table n°1 et n°2)

Asthma in children is highly heterogeneous and is related not only to lung function and atopy but also to systemic inflammation. Another TAP cohort recruited 309 children of school age and identified three independent clusters of asthma in a cluster analysis applied to 15 variables[44].

The "*Asthma with multiple allergic sensitizations"* cluster was more atopic, with more frequent severe exacerbations but relatively normal lung function. This type of severe asthma is associated with an inflammation predominantly of "allergic type" (with eosinophil and basophil cells) in combination with multiple allergic sensitizations and elevated total IgE. Many studies, mainly in children, have confirmed that asthma at risk of severe exacerbations or difficult to control is associated with allergic asthma[45].

The "*Severe asthma with bronchial obstruction*" cluster was characterized by the lowest lung function and more blood neutrophils, IgG and IgA but less atopy. The children were also older and had a higher BMI. Other studies have shown an association between the severity of asthma and neutrophilic inflammation detected by induced sputum in particular in adult[4647] The TAP results underline that neutrophilic

asthma exists also in children with unknown aetiology[38]. This finding supports those of the Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy (EGEA). The EGEA family sample consisted of 388 French nuclear families that included 253 families ascertained through offspring with asthma (one offspring proband in 90% of families and two in the remainder) and 135 families ascertained through a parent with asthma. EGEA asthma phenotypes showed that the phenotype of "active treated allergic childhood-onset asthma" is associated with blood eosinophilia, while "active treated adult-onset asthma" is associated to blood neutrophils"[48]. Another group linked inflammation to asthma severity. The Severe Asthma Research Program (SARP) funded by the The National Heart, Lung, and Blood Institute (NHLBI) recruited subjects with asthma of all ages who met the ATS workshop definition of severe asthma. An additional group of subjects with asthma that did not meet the criteria for severe asthma (not severe) was also studied. In the same manner as in previous studies, a multivariate approach identified a severity spectrum related to sputum cellular inflammation from mild-to moderate allergic asthma with minimal or eosinophil predominant inflammation to moderate-to-severe asthma with neutrophil-predominant or mixed granulocytic inflammation[49].

Allergic asthma is a heterogeneous disease (Table n°2)

Allergy expression defines the prognosis of asthma during childhood but the problem is how should atopy be defined? Atopy depends on allergic sensitization (single or multiple but also the type of allergen and the date of onset of sensitization) and the association with allergic comorbidities (AR, eczema, food allergy).

In a recent article[16], a TAP cohort study performed in 125 children of school age with allergic asthma described four phenotypes.

The "*House dust mite (HDM) Sensitization and Mild Asthma*" phenotype, 98% of whom were monosensitized and had mild asthma (74% of cases). In a previous study, we had described a similar phenotype of asthma with few allergic sensitizations and mild asthma[38]. Moore *et al.*[50] also defined a large cluster (82% of the study population) of adults with early-onset mild atopic asthma. The Childhood Asthma Prevention Study (CAPS)[7] included pregnant women whose unborn children were at high risk of developing asthma because of a parent or a sibling with a current diagnosis of asthma or frequent wheeze recruited from the antenatal clinics of six hospitals in Sydney, Australia. Six hundred and sixteen women were enrolled in the main trial between September 1997 and December 1999[51] Both the CAPS and MAAS cohorts[30] as well as the TAP cohort defined an HDM monosensitized population which confers a better prognosis of asthma than children with multiple sensitizations.

In the TAP cohort, the "*Pollen Sensitization with Severe Exacerbations*" phenotype comprised 92% of children with severe exacerbations and pollen sensitization. Erbas *et al.*[52] showed a linear increase in asthma emergency department presentations correlated with an increased concentration of ambient grain of grass pollen ($p< 0.001$). More specifically, pollen-allergic children seem to be admitted due to food-induced anaphylaxis more often during the pollen season ($p = 0.015$) [5354].

In the TAP cohort "*Multiple Allergies and Severe Asthma*" phenotype includes children with a multiple allergic phenotype (100% of the children had eczema and multiple sensitizations) and higher values of IgE (1123 kU/L) and FeNO (67 ppb). Moreover, the severity of asthma is attested by the highest proportion of moderate to severe asthma (95%) and a significant decrease in FEF_{25-75}. The clinical relevance of FeNO in asthma severity was also identified in the PIAMA cohort[55] and by Sonnappa S *et al* [56]: FeNO measured at 8 years was associated with persistent, intermediate, and

late-onset wheeze only in children with allergic sensitization at 8 years. The German Multicentre Allergy Study (MAS) cohort consists of 1,314 children born in 1990 and followed at the ages of 1, 3, 6, 12, 18, and 24 months and then at yearly intervals thereafter until age 13 years[57]. This cohort found that eczema associated with filaggrin loss-of-function mutations conveyed a greater risk of severe asthma phenotype with a significant decrease in pulmonary function at puberty. Moreover, in accordance with the TAP results, children (especially boys) with persistent wheeze, frequent asthma exacerbations, and multiple early atopy in the MAAS cohort[25] had diminished lung function throughout childhood and were at higher risk of a progressive loss of lung function from age 3 to 11 years. Furthermore, Garden *et al.* (the CAPS cohort)[47] showed that asthma risk is related to the type of allergen sensitization, with a strong association between the mixed food and inhalant sensitization class and poor asthma control at age 8 years.

Finally the last phenotype discovered in the TAP cohort was the "*Multiple Allergic Sensitizations and Mild Asthma*" phenotype, in which 97% of the children had multiple sensitizations and 100% mild asthma. Several reasons could explain this novel result linking multiple sensitizations and mild asthma severity. Firstly, this phenotype is associated with a low percentage of eczema and it is known that eczema plus allergic asthma confers an intrinsically severe asthma. Secondly, multiple allergic sensitizations could be related to biological sensitizations rather than to real allergy explaining why total IgE and FeNO values were lower in this group.

To summarize, severe allergic asthma in children could consist of two phenotypes depending on the type of sensitization and the association with other allergic comorbidities: severe phenotype with acute exacerbations and related to pollens and/or

food sensitizations and a severe phenotype with very high inflammatory markers constantly associated with atopic dermatitis.

Asthma phenotypes and* p*ersonalized medicine

The Childhood Asthma Management Program (CAMP) is a multicenter, randomized, double-masked clinical trial designed to explore the clinical relevance of the clustering approach in assessing the long-term effects of three inhaled treatments for mild to moderate childhood asthma: budesonide (a glucocorticoid used daily) and albuterol (a short-acting β-agonist bronchodilator used as needed); nedocromil (a nonsteroid anti-inflammatory agent used daily) and albuterol; and placebo and albuterol[11]. Overall, in this cohort Howrylak *et al.*[58] found five reproducible patient clusters that could be differentiated on the basis of atopic burden, degree of airway obstruction, and history of exacerbation. Moreover, the clustering approach predicted long-term asthma control as well as longitudinal differences in pulmonary function and response to long term treatment.

Conclusions

As already suggested[59], our analysis confirms that existing cohorts studies have provided data useful for the ascertainment of early life asthma phenotypes. Further studies should be designed to find noninvasive biological markers to better define wheezing phenotypes in very young children associated with an elevated risk of developing asthma to define personalized medicine based on targeted treatment.

FIG 1: Representation of the three clusters according to the percentage of Multiple Trigger Wheeze, Severity and Atopy for the entire population (n=551)

MTW (Multiple Trigger Wheeze) is defined as wheezing during colds and with other triggers such as house dust, grass, pets, tobacco smoke, exercise or cold air; Severity is defined as percentage of moderate to severe asthma according to GINA classification; Circled area represents the percentage of atopy defined as percentage of positive Phadiatop Infant® ≥ 0.35U/ml. (Ref 14)

FIG 2: Classification tree for entire population using two variables i.e. asthma severity according to GINA classification and Phadiatop Infant®

Subjects are assigned to the three clusters that range from milder recurrent wheeze (Cluster 1) to more severe disease (Clusters 2 and 3) with 76% of the subject assigned to the appropriate cluster. Tree performances are given. (Ref 14)

FIG 3: Change of wheeze phenotypes from under 3 years until 5 years of age

Y axis represents change (expressed in percentage) of separate 3 clusters towards the 4 clusters at 5 years of age. At 3 years of age : *Mild EVW*: Episodic Viral Wheeze (wheezing only during colds and asymptomatic between episodes), Atopic *MTW:* Multiple Trigger Wheeze (wheezing during colds and with other triggers such as house dust, grass, pets, tobacco smoke, exercise or cold air), *NAUW:* Non-atopic Uncontrolled Wheeze. The Fisher exact test rejects the independence assumption ($p<0.001$). (Ref 17)

325 **Table n° 1: Asthma phenotypes in very young children**

326

	Gender Predominance	**High risk of exacerbation**	**Prognosis in term of asthma persistence during childhood**	**Predominant inflammation /Response to corticosteroid**
Mild Episodic Viral Wheeze	**Male** [18]	**No** [14,15,18]	**Good** [17,20,21,31]	**None**[14] **/High**[14]
Atopic Multiple-Trigger	**Male**[14,21,25]	**Yes**[25,14,18]	**Poor**[14,21,17,,22,19,25,28,29 33,34,35,36]	**Eosinophilic**[14,18,15,19] **/Intermediate**[14]
Nonatopic wheeze phenotype	**Female**[14,26]	**No**[14,18,15]	**Intermediate (depending of late onset of allergic expression)** [17,21]	**Neutrophilic ? /Low**[14]

327

328

329 **Table n° 2: Asthma phenotypes in children at school age**

330

	Gender Predominance	Age of asthma onset	High Risk of exacerbation	Prognosis in tem of lung function decline	Predominant inflammation /Response to corticosteroid
House dust mite Sensitization and Mild Asthma	**M**	**Early** [16,46,30]	_	_	**Eosinophil /High**[16]
Pollen Sensitization with Severe Exacerbations"	**M**	**Late** [16]	**Yes**[16,39]	_	**Eosinophil** [16,,46,47,48] **/High**[16]
Multiple Allergic Sensitizations and eczema associated with Severe Asthma	**M**	**Early**[16,25,38,51]	_	**Poor** [16,51,45]	**High Eosinophil** [16,38,49] **/Low** [16]
Multiple Allergic Sensitizations and Mild Asthma	**M**	**Early**[16]	_	_	**Eosinophil** [16] **/High** [16]
Severe asthma with bronchial obstruction	**F**	**Late** [38,42,43,44]	_	**Poor** [43,44]	**Neutrophil** [38] **/Very low** [38,42,44]

331

332

References

[1] Eder W, Ege MJ, von Mutius E. The asthma epidemic. N Engl J Med 2006;355:2226–35.

[2] McCallister JW. Reslizumab and eosinophilic asthma: one step closer to phenotype-directed therapy? Am J Respir Crit Care Med 2011;184:1096–7.

[3] Phelan PD, Robertson CF, Olinsky A. The Melbourne Asthma Study: 1964-1999. J Allergy Clin Immunol. 2002 Feb;109(2):189-94.

[4] Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. Lancet 2008;372:1107–19.

[5] Busse WW, Morgan WJ, Gergen PJ, et al. Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. N Engl J Med 2011 ;364 : 1015-15.

[6] A plea to abandon asthma as a disease concept. Lancet 2006;368:705.

[7] Arshad SH, Stevens M, Hide DW. The effect of genetic and environmental factors on the prevalence of allergic disorders at the age of two years. Clin Exp Allergy 1993; 23: 504-511.

[8] Bergmann RL, Bergmann KE, Lau-Schadendorf S, Luck W, Dannemann A, Bauer CP. Atopic diseases in infancy. The German multicenter atopy study (MAS-90) Pediatr Allergy Immunol. 1995; 5:19-25.

[9] Custovic A, Simpson BM, Murray CS, Lowe L, Woodcock A. The national asthma campaign manchester asthma and allergy study. Pediatr Allergy Immunol 2002; 13: 32-37.

[10] Brunekreef B, Smit J, de Jongste J, Neijens H, Gerritsen J, Postma D, Aalberse R, Koopman L, Kerkhof M, Wilga A, van Strien R. The prevention and incidence of asthma and mite allergy (PIAMA) birth cohort study: design and first results. Pediatr Allergy Immunol. 2002; 13: 55-60.

[11] Clarisse B, Nikasinovic L, Poinsard R, Just J, Momas I. The Paris prospective birth cohort study: which design and who participates? Eur J Epidemiol. 2007; 22: 203-210.

[12] Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J, Molloy L, Ness A, Ring S, Davey Smith G. Cohort Profile: the 'children of the 90s'-the index

offspring of the Avon Longitudinal Study of Parents and Children. Int J Epidemiol 2013; 42: 111-127.

[13] Mihrshahi S, Peat J, Webb K, Tovey E, Marks G, Mellis C, Leeder SR. The Childhood Asthma Prevention Study (CAPS): design and research protocol of a large randomised trial of the primary prevention of asthma. Control Clin Trials 2001; 22:333-54.

[14] Spycher BD, Silverman M, Pescatore AM, Beardsmore CS, Kuehni CE. Comparison of phenotypes of childhood wheeze and cough in 2 independent cohorts J Allergy Clin Immunol 2013; 132: 1058-1067.

[15] Hovland V, Riiser A, Mowinckel P, Carlsen KH, Lødrup Carlsen KC. The significance of early recurrent wheeze for asthma outcomes in late childhood. Eur Respir J. 2013; 41: 838-845.

[16] Kauffmann F, Dizier MH. EGEA (Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy)-design issues. EGEA Co-operative Group. Clin Exp Allergy. 1995; 25: 19-22.

[17] The Childhood Asthma Management Program (CAMP): design, rationale, and methods. Childhood Asthma Management Program Research Group. Control Clin Trials. 1999; 20: 91-120.

[18] Dolan CM, Fraher KE, Bleecker ER, Borish L, Chipps B, Hayden ML, Weiss S, Zheng B, Johnson C, Wenzel S; TENOR Study Group. Design and baseline characteristics of the epidemiology and natural history of asthma: Outcomes and Treatment Regimens (TENOR) study: a large cohort of patients with severe or difficult-to-treat asthma. Ann Allergy Asthma Immunol. 2004; 92: 32-39.

[19] American Thoracic Society Workshop. Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. Am J Respir Crit Care Med 2000;162: 2341–2351.

[20] Just J, Gouvis-Echraghi R, Couderc R, Guillemot-Lambert N, Saint-Pierre P. Two novel severe wheezy young children phenotypes: boys atopic multiple-trigger and girls non-atopic uncontrolled wheeze. J Allergy Clin Immunol 2012; 130: 103-110.

[21] Gouvis-Echraghi R, Saint-Pierre P, Besharaty AA, Bernard A, Just J.Exhaled nitric oxide measurement confirms 2 severe wheeze phenotypes in young children from the Trousseau Asthma Program. J Allergy Clin Immunol. 2012; 130:1005-1007.

[22] Just J, Saint-Pierre P, Gouvis-Echraghi R, Laoudi Y, Roufai L, Momas I, Annesi Maesano I.Childhood allergic asthma is not a single phenotype. J Pediatr. 2014; 164: 815-820.

[23] Just J, Saint-Pierre P, Gouvis-Echraghi R, Boutin B, Panayotopoulos V, Chebahi N, Ousidhoum-Zidi A, Khau CA. Wheeze phenotypes in young children have different courses during the preschool period. Ann Allergy Asthma Immunol. 2013; 111: 256-261.

[24] Herr M, Just J, Nikasinovic L, Foucault C, Le Marec AM, Giordanella JP, Momas I. Risk factors and characteristics of respiratory and allergic phenotypes in early childhood. J Allergy Clin Immunol 2012; 130:389-96.

[25] Taussig LM, Wright AL, Morgan WJ, Harrison HR, Ray CG, Group Health Medical Associates. The Tucson Children's Respiratory Study. I. Design and implementation of a prospective study of acute and chronic respiratory illness in children. Am J Epidemiol 1989;129: 1219-1231.

[26] Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. N Engl J Med 1995; 332: 133-138.

[27] Spycher BD, Silverman M, Brooke AM, Minder CE, Kuehni CE. Distinguishing phenotypes of childhood wheeze and cough using latent class analysis. Eur Respir J. 2008; 31: 974-81.

[28]Henderson J, Granell R, Heron J, Sherriff A, Simpson A, Woodcock A, Strachan DP, Shaheen SO, Sterne JA. Associations of wheezing phenotypes in the first 6 years of

life with atopy, lung function and airway responsiveness in mid-childhood. Thorax 2008; 63: 974-980.

[29] Mandhane PJ, Greene JM, Cowan JO, Taylor DR, Sears MR. Sex differences in factors associated with childhood- and adolescent-onset wheeze. Am J Respir Crit Care Med 2005; 172: 45-54.

[30] Sears MR, Burrows B, Flannery EM, Herbison GP, Holdaway MD. Atopy in childhood. I. Gender and allergen related risks for development of hay fever and asthma. Clin Exp Allergy 1993; 23: 941-948.

[31] Belgrave DC, Buchan I, Bishop C, Lowe L, Simpson A, Custovic A. Trajectories of lung function during childhood. Am J Respir Crit Care Med. 2014; 189: 1101-1109.

[32] Isozaki A, Shoda T, Mimura S, Ogawa N, Noma T, Kawano T, Nakamura Y, Kawano Y. A trial of hierarchical cluster analysis of hospitalized wheezy infants and young children based on clinical factors. Arerugi 2011; 60: 586-592.

[33] Rackemann FM, Mallory TB. Intrinsic Asthma. Trans Am Clin Climatol Assoc 1941; 57: 60-73.

[34] Jose A. Castro-Rodriguez, Lorena Cifuentes, Carlos E. Rodríguez-Martínez. The asthma predictive index remains a useful tool to predict asthma in young children with recurrent wheeze in clinical practice Journal of Allergy and Clinical Immunology 2011; 127: 1082-1083.

[35] Leonardi NA, Spycher BD, Strippoli MP, Frey U, Silverman M, Kuehni CE. Validation of the Asthma Predictive Index and comparison with simpler clinical prediction rules. J Allergy Clin Immunol 2011; 127: 1466-1472.

[36] Matricardi PM, Illi S, Keil T, Wagner P, Wahn U, Lau S. Predicting persistence of wheezing: one algorithm does not fit all. Eur Respir J 2010;35:701-3.

[37] Piippo-Savolainen E, Korppi M. Wheezy babies--wheezy adults? Review on long-term outcome until adulthood after early childhood wheezing. Acta Paediatr 2008; 97: 5-11.

[38] Stern DA, Morgan WJ, Wright AL, Guerra S, Martinez FD. Poor airway function in early infancy and lung function by age 22 years: a non-selective longitudinal cohort study.Lancet. 2007; 370(9589):758-64.

[39] Illi S, von Mutius E, Lau S, Niggemann B, Gruber C, Wahn U. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. Lancet 2006; 368:763-70.

[40] Kusel MM, Kebadze T, Johnston SL, Holt PG, Sly PD. Febrile respiratory illnesses in infancy and atopy are risk factors for persistent asthma and wheeze. Eur Respir J. 2012;39(4):876-82.

[41] Simpson A, Tan VYF, Winn J, Svensén M, Bishop CM, Heckerman DE, Buchan I, Custovic A. Beyond atopy: multiple patterns of sensitization in relation to asthma in a birth cohort study. Am J Respir Crit Care Med 2010; 181:1200-1206.

[42] Lazic N, Roberts G, Custovic A, Belgrave D, Bishop CM, Winn J, Curtin JA, Hasan Arshad S, Simpson A.Multiple atopy phenotypes and their associations with asthma: similar findings from two birth cohorts. Allergy. 2013; 68: 764-70.

[43] From the Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2009. Available from: http://www.ginasthma.org, Date last update june 9 2014. Date last accessed June 9 2014

[44] Just J, Gouvis-Echraghi R, Rouve S, Wanin S, Moreau D, Annesi-Maesano I. Two novel, severe asthma phenotypes identified during childhood using a clustering approach. Eur Respir J 2012; 40: 55-60.

[45] Carroll WD, Lenney W, Child F, Strange RC, Jones PW, Whyte MK, Primhak RA, Fryer AA. Asthma severity and atopy: how clear is the relationship? Arch Dis Child 2006; 91: 405-409.

[46] Wang F, He XY, Baines KJ, Gunawardhana LP, Simpson JL, Li F, Gibson PG. Different inflammatory phenotypes in adults and children with acute asthma. Eur Respir J 2011; 38: 567-574.

[47] The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. European Network for Understanding Mechanisms of Severe Asthma. Eur Respir J 2003; 22: 470-477.

[48] Siroux V, Basagaña X, Boudier A, Pin I, Garcia-Aymerich J, Vesin A, Slama R, Jarvis D, Anto JM, Kauffmann F, Sunyer J. Identifying adult asthma phenotypes using a clustering approach. Eur Respir J. 2011; 38: 310-317.

[49] Moore WC, Hastie AT, Li X, Li H, Busse WW, Jarjour NN, Wenzel SE, Peters SP, Meyers DA, Bleecker ER; National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis.J Allergy Clin Immunol. 2014; 133: 1557-1563.

[50] Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, D'Agostino R Jr, Castro M, Curran-Everett D, Fitzpatrick AM, Gaston B, Jarjour NN, Sorkness R, Calhoun WJ, Chung KF, Comhair SA, Dweik RA, Israel E, Peters SP, Busse WW, Erzurum SC, Bleecker ER; National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med 2010; 181: 315-323.

[51] Garden FL, Simpson JM, Marks GB; CAPS Investigators. Atopy phenotypes in the Childhood Asthma Prevention Study (CAPS) cohort and the relationship with allergic disease: clinical mechanisms in allergic disease. Clin Exp Allergy. 2013; 43: 633-641.

[52] Erbas B, Akram M, Dharmage SC, Tham R, Dennekamp M, Newbigin E, Taylor P, Tang ML, Abramson MJ. The role of seasonal grass pollen on childhood asthma emergency department presentations. Clin Exp Allergy 2012; 42: 799-805.

[53] Vetander M, Helander D, Flodström C, Ostblom E, Alfvén T, Ly DH, Hedlin G, Lilja G, Nilsson C, Wickman M. Anaphylaxis and reactions to foods in children--a population-based case study of emergency department visits. Clin Exp Allergy 2012; 42: 568-577.

[54]Katelaris CH. Food allergy and oral allergy or pollen-food syndrome. Curr Opin Allergy Clin Immunol 2010; 10: 246-251.

[55] van der Valk RJ, Caudri D, Savenije O, Koppelman GH, Smit HA, Wijga AH, Postma DS, Kerkhof M, Brunekreef B, de Jongste JC. Childhood wheezing phenotypes and FeNO in atopic children at age 8. Clin Exp Allergy. 2012; 42: 1329-1336.

[56] Sonnappa S, Bastardo CM, Saglani S, Bush A, Aurora P. Relationship between past airway pathology and current lung function in preschool wheezers. Eur Respir J. 2011; 38: 1431-1436.

[57] Marenholz I, Kerscher T, Bauerfeind A, Esparza-Gordillo J, Nickel R, Keil T, Lau S, Rohde K, Wahn U, Lee YA. An interaction between filaggrin mutations and early food sensitization improves the prediction of childhood asthma. J Allergy Clin Immunol 2009; 123: 911-916.

[58] Howrylak JA, Fuhlbrigge AL, Strunk RC, Zeiger RS, Weiss ST, Raby BA; Childhood Asthma Management Program Research Group. Classification of childhood asthma phenotypes and long-term clinical responses to inhaled anti-inflammatory medications. J Allergy Clin Immunol. 2014; 133: 1289-1300.

[59] Bousquet J, Gern JE, Martinez FD, Anto JM, Johnson CC, Holt PG, Lemanske RF Jr, Le Souëf PN, Tepper RS, von Mutius ER, Arshad SH, Bacharier LB, Becker A, Belanger K, Bergström A, Bernstein DI, Cabana MD, Carroll KN, Castro M, Cooper PJ, Gillman MW, Gold DR, Henderson J, Heinrich J, Hong SJ, Jackson DJ, Keil T, Kozyrskyj AL, Lødrup Carlsen KC, Miller RL, Momas I, Morgan WJ, Noel P, Ownby DR, Pinart M, Ryan PH, Schwaninger JM, Sears MR, Simpson A, Smit HA, Stern DA, Subbarao P, Valenta R, Wang X, Weiss ST, Wood R, Wright AL, Wright RJ, Togias A, Gergen PJ. Birth cohorts in asthma and allergic diseases: report of a NIAID/NHLBI/MeDALL joint workshop. J Allergy Clin Immunol. 2014; 133: 1535-1546.

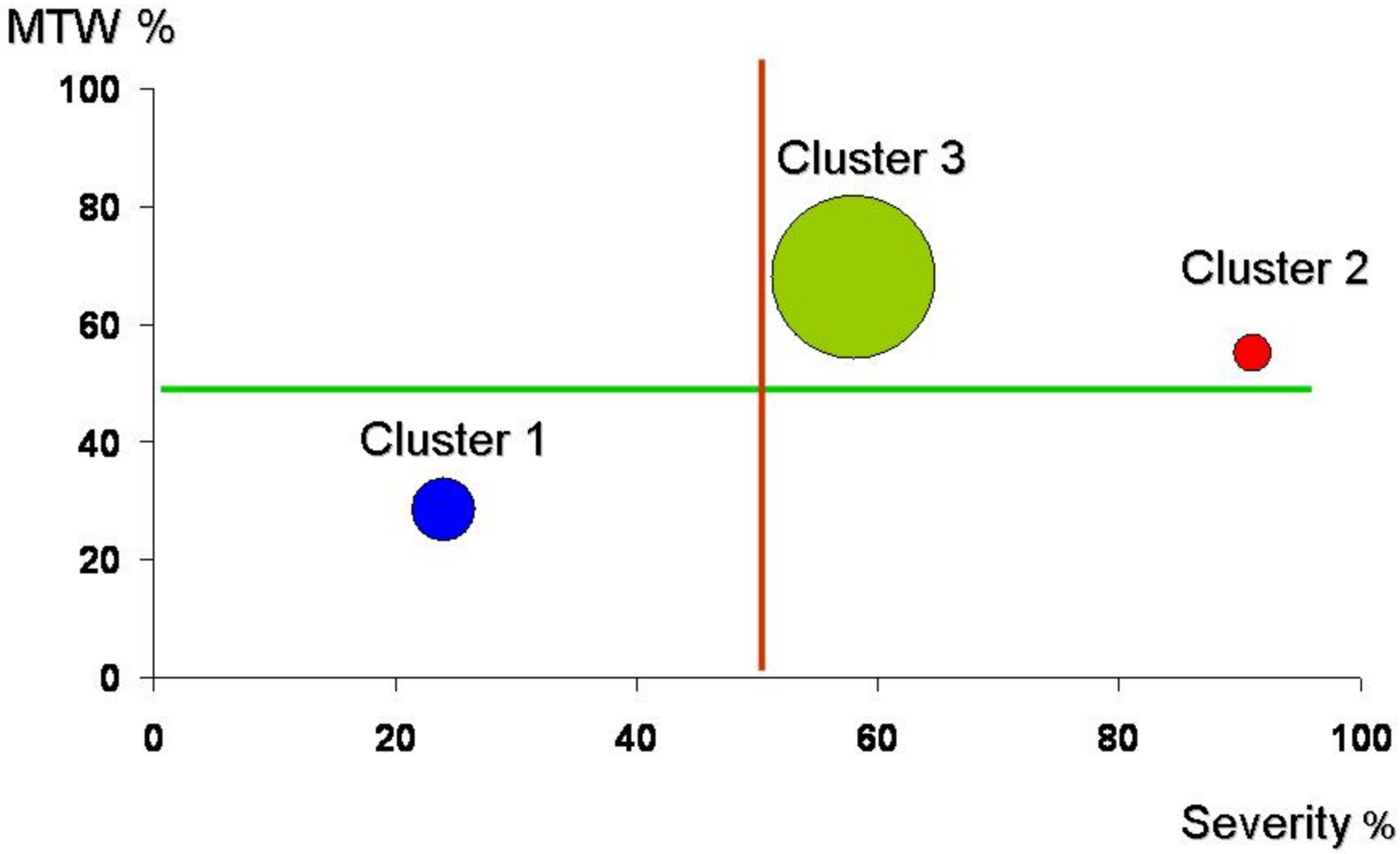
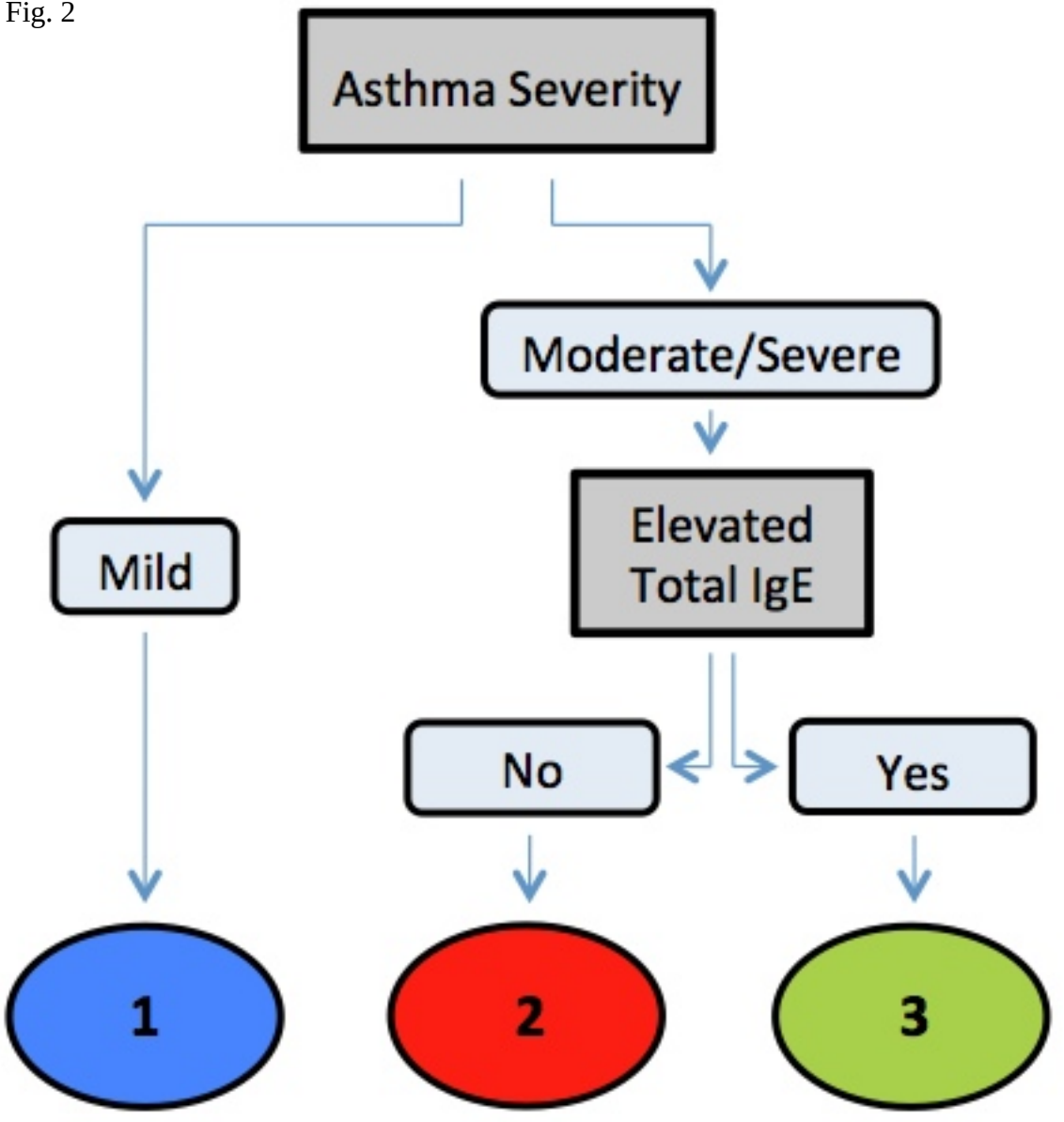

MTW %
100
80
60
40
20
0
Cluster 3
Cluster 2
Cluster 1
0
20
40
60
80
100
Severity %

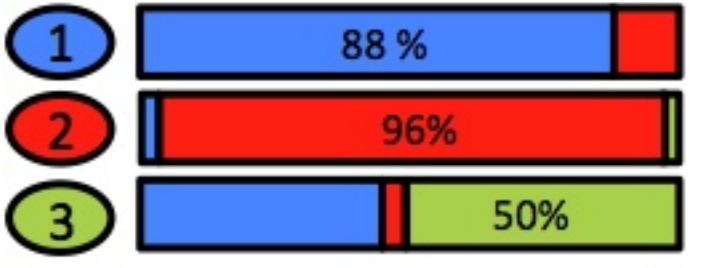
Fig. 1

Fig. 2



Population		Prediction		
		1	2	3
Cluster label	1	**70**	10	0
	2	1	**53**	1
	3	14	2	**16**

Percentage		Prediction		
		1	2	3
Cluster label	1	**0.88**	0.12	0
	2	0.02	**0.96**	0.02
	3	0.44	0.06	**0.5**



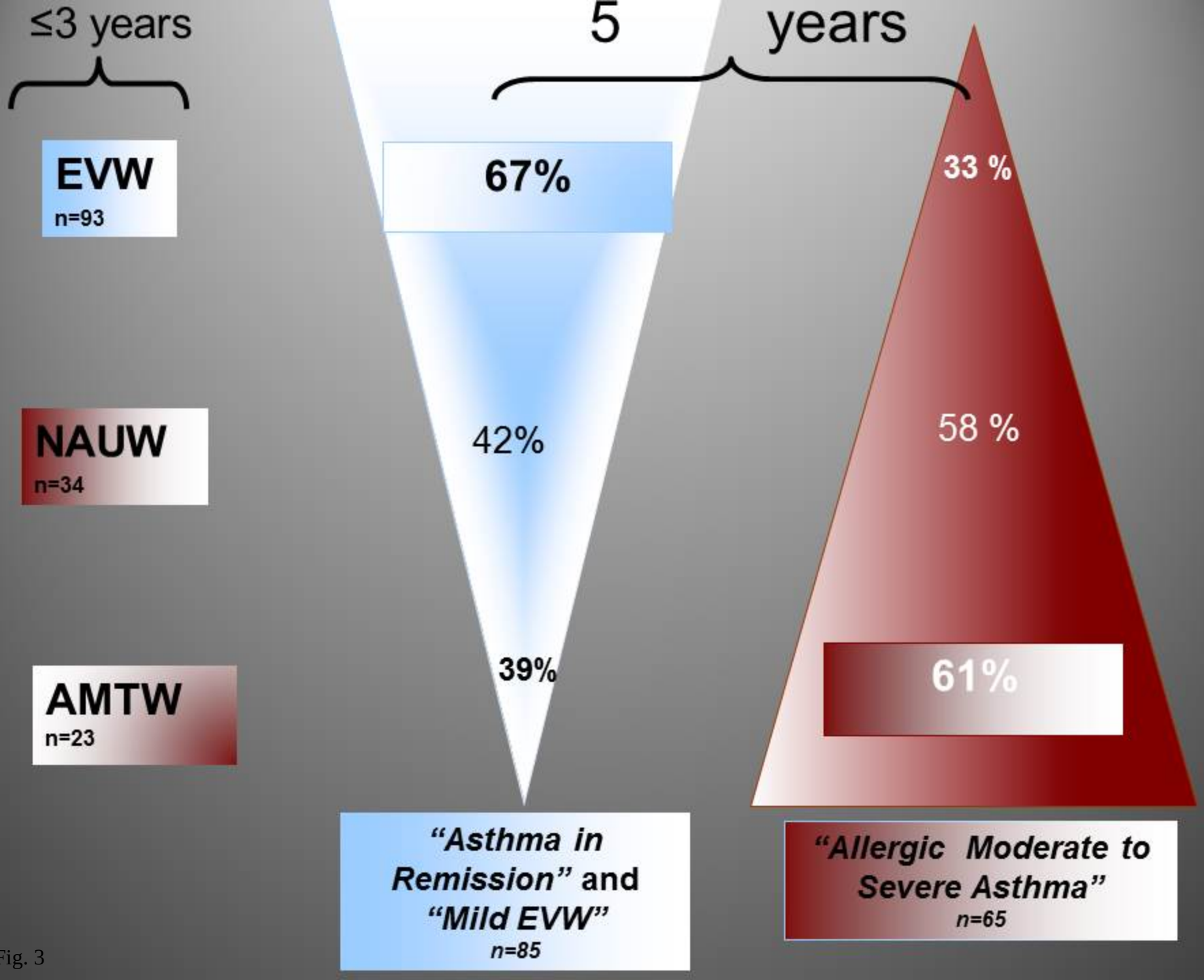

≤3 years
5
years
EVW
n=93
67%
33 %
NAUW
n=34
42%
58 %
AMTW
n=23
39%
61%
"Asthma in Remission" and "Mild EVW"
n=85
"Allergic Moderate to Severe Asthma"
n=65

Fig. 3