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## **New Perspectives on Difficult Asthma; Sex and Age of Asthma-Onset Based Phenotypes**

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HAL Authorization

1 **Title: New Perspectives on Difficult Asthma; Sex and Age of Asthma-Onset Based**

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47 **ABSTRACT:**

48 **Background:** Asthma is a diverse condition that differs with age and sex. However, it  
49 remains unclear how sex, age of asthma-onset, and/or their interaction, influence clinical  
50 expression of more problematic adult “difficult” asthma.

51 **Objectives:** To better understand the clinical features of difficult asthma within a real-world  
52 clinical setting using novel phenotypic classification, stratifying subjects by sex and age of  
53 asthma-onset.

54 **Methods:** Participants in a longitudinal difficult asthma clinical cohort study (Wessex  
55 AsThma CoHort of difficult asthma; WATCH), United Kingdom, (n=501) were stratified  
56 into 4 difficult asthma phenotypes based on sex and age of asthma-onset (early<18-years or  
57 adult $\geq$ 18-years) and characterised in relation to clinical and pathophysiological features.

58 **Results:** The cohort had more female participants (65%) but had similar proportions of  
59 participants with early or adult-onset disease. Early-onset female disease was commonest  
60 (35%), highly atopic, with good spirometry and strong associations to some physical  
61 comorbidities but highest psychophysiologic comorbidities. Adult-onset females also had  
62 considerable psychophysiologic comorbidities, highest obesity, and were least atopic.  
63 Amongst male subjects, proportionately more had adult-onset disease. Early-onset male  
64 disease was rarest (14%) but associated with worst lung function, high smoking, atopy and  
65 fungal sensitisation. Despite shortest disease duration, adult-onset males had highest use of  
66 maintenance oral corticosteroid, poor lung function and highest FeNO in spite of highest  
67 smoking prevalence.

68 **Conclusion:** This study shows that sex, age of asthma-onset, and their interactions influence  
69 different clinical manifestations of difficult asthma and identifies a greater risk for lung

70 function loss and oral corticosteroid dependency associated with smoking in adult-onset male  
 71 subjects.

72 **Hig**

- **What is already known about this topic?**

Asthma shows differing sex associations across the life-course, with male predominance in childhood switching to female predominance in adulthood. How such disease associations relate to more difficult asthma in adulthood is unclear.

- **What does this article add to our knowledge?**

This real-world study shows that stratifying difficult asthma by sex and age of asthma-onset identifies clinically important phenotypes that are currently poorly recognised including more common early-onset female, and more severe adult-onset male, difficult asthma.

- **How does this study impact current management guidelines?**

This study describes clinical features of difficult asthma from a new phenotypic perspective. By characterising these novel phenotypes, and their multimorbid nature, this study can guide better identification and management of patients with difficult asthma.

**hli****ght****s****Bo****x:****K****E****Y****W****O****R****D****S**

89 : age of onset, comorbidity, difficult asthma, lung function, phenotypes, sex, smoking

90

91 **ABBREVIATIONS:**

92 ABPA Allergic Bronchopulmonary Aspergillosis

93 ACQ Asthma Control Questionnaire

94 BD Bronchodilator

95 BDP Beclomethasone

96 BMI Body Mass Index

97 CF Cystic Fibrosis

98 COPD Chronic Obstructive Pulmonary Disease

99 DLCO Diffusing Capacity of Lung for Carbon Monoxide

100 EGPA Eosinophilic Granulomatosis with Polyangitis

101 FeNO Fractional Exhaled Nitrogen Oxide

102 FEF 25-75% Forced expiratory flow between 25-75% exhalation

103 FEV1 Forced Expiratory Volume in 1 second

104 FVC Forced Vital Capacity

105 GORD Gastroesophageal reflux

106 ILO Intermittent Laryngeal Dysfunction

107 HADS Hospital Anxiety and Depression Score

108 HRCT High resolution computed tomography

109 ICU Intensive Care Unit

110 IgE Immunoglobulin E

111 OCS Oral corticosteroids

112 KCO Diffusing Capacity of Lung for Carbon Monoxide corrected for alveolar volume

113 SAFS Severe Asthma with Fungal Sensitisation

114 SPT Skin prick test

115 SNOT 22 Sinonasal Outcome Test

**116 INTRODUCTION**

117 Asthma is a diverse condition with different characteristics across the life-course. It is more  
118 prevalent and problematic in males during childhood but following puberty becomes a  
119 predominantly female symptomatic disease in adulthood<sup>1-5</sup>. Potential contributing factors to  
120 these age-related changing sex associations include remission of childhood asthma in boys  
121 and asthma development in adolescent girls<sup>4</sup>. Postulated mechanisms for these processes  
122 include protective effects of male (androgenic) sex hormones and potentially sex-specific  
123 deleterious effects of female sex hormones<sup>6-8</sup>. Such concepts are supported by changes in  
124 asthma prevalence with menarche<sup>8-9</sup> and menopause<sup>8-10</sup>. Additional important contributory  
125 mechanisms include differences in innate and adaptive immune responses in males and  
126 females during reproductive life<sup>11</sup>. Other environmental or lifestyle factors, like smoking  
127 exposure/habit, may plausibly influence these disease patterns too<sup>5</sup>. In that context, some  
128 studies have demonstrated associations between smoking and asthma in females but not  
129 males<sup>12</sup>, while others have associated older onset asthma with male smokers<sup>13</sup>. Conversely,  
130 obesity shows clear association to incident asthma in females across the life-course<sup>14</sup>.

131 Studies have shown both childhood and adult-onset patterns of adult severe asthma<sup>15-18</sup>, but it  
132 is widely regarded that adult severe asthma is commoner in females, with severe asthma  
133 cohort studies consistently showing female predominance<sup>15-18</sup>. Contrary to traditional  
134 understanding of asthma-sex associations across the life-course, studies also point to both  
135 clusters of childhood-onset female asthma and adult-onset male severe asthma<sup>5</sup>. However, it  
136 remains unclear how sex and age of asthma-onset influence models of the more commonly  
137 encountered clinical challenge of adult difficult asthma.

138 Most asthmatics can be treated effectively but 5-10% suffer from “difficult asthma”, with  
139 poor disease control and disproportionately high socio-economic costs<sup>19-21</sup>. Difficult asthma  
140 describes asthma in which co-morbidities, inadequate treatment, suboptimal inhaler technique

141 and/or poor adherence impede good asthma control. This broad definition also encompasses a  
142 subset of patients with truly severe, treatment refractory, asthma that remain sub-optimally  
143 controlled despite optimised treatment of both asthma and contributory factors<sup>19-24</sup>. Better  
144 insight of how sex and age of asthma-onset relate to difficult asthma could support more  
145 personalised characterisation of that problematic condition, offering a path to better  
146 outcomes. Here we describe influences of sex and age of onset on the nature of adult difficult  
147 asthma within a clinical cohort; the Wessex AsThma CoHort of difficult asthma (WATCH)  
148 Study.

149

**150 METHODS****151 WATCH Data Collection**

152 WATCH is an ongoing clinical cohort of patients with difficult asthma based at University  
153 Hospitals Southampton NHS Foundation Trust (UHSFT), Southampton, United Kingdom  
154 (UK). All patients managed with British Thoracic Society Step “high dose therapies” and/or  
155 “continuous or frequent use of oral corticosteroids”<sup>25</sup> in the Adult or Transitional Regional  
156 Asthma Clinic at UHSFT are invited to participate. Briefly, research data capture is aligned  
157 with the extensive clinical characterisation required of a commissioned National Health  
158 Service (NHS) Specialist Centre for Severe Asthma<sup>26</sup>. Data acquisition at enrolment includes  
159 detailed clinical, health and disease-related questionnaires, anthropometry, allergy skin prick  
160 testing (SPT), lung function testing, radiological imaging (in a subset) and collection of  
161 biological samples (blood, and urine). Brief longitudinal updates of data are obtained  
162 annually. A detailed outline of study protocol and methodology is published elsewhere<sup>27</sup>. The  
163 study was approved by West Midlands – Solihull Research Ethics Committee (REC  
164 reference: 14/WM/1226). Here, we present data at enrolment from the first 501 WATCH  
165 patients.

**166 Statistical Analysis**

167 Statistical analysis was performed using SPSS 25 (NY, USA), GraphPad Prism 7 (La Jolla  
168 California, USA) and R (Vienna, Austria). Quantitative variables are presented as mean plus  
169 standard deviation and categorical variables as frequencies (percentages).

170 We defined four phenotypes using two binary variables: sex (male, female) and age of  
171 asthma diagnosis (early <18years, adult  $\geq$ 18 years). Age of asthma diagnosis was highly  
172 correlated with age of reported disease onset (see online repository text). The interaction  
173 between sex and age of diagnosis for quantitative variables was tested with a two-way  
174 ANOVA. If the interaction was significant, a one-way ANOVA was applied to each factor  
175 separately. The same strategy was applied to both count and binary variables but using

176 negative binomial models and logistic models, respectively. These analyses were realised  
177 with R software, using the *glm* function for ANOVA and logistic regression models and  
178 *glm.nb* for negative binomial models. Data modelling checks were undertaken for model  
179 verification. If the one-factor model was better than two-factors model (by stepwise  
180 selection), the significant effect was shown from the one-factor model. Significant effects are  
181 presented as a difference of means for quantitative variables, rate ratio for count variables,  
182 and odds ratio for binary variables, each with 95% confidence intervals. A p value of <0.05  
183 was regarded statistically significant.

184

**185 RESULTS****186 Representativeness of WATCH Cohort to the Difficult Asthma Clinic**

187 Patients not enrolled in WATCH but attending the UHSFT Difficult Asthma clinic were  
188 compared to enrolment data from the WATCH cohort (online repository text; Table E1).  
189 There were no statistically significant differences between WATCH and non-WATCH  
190 patients for sex or smoking status but median age of WATCH patients was 1-year younger.  
191 There was no statistically significant difference for patients receiving biological therapies but  
192 maintenance oral corticosteroid (OCS) use was significantly higher in WATCH patients.

**193 WATCH Cohort Description**

194 Of 501 cohort subjects, 2/3 (65.3%) were female with a mean study enrolment age of 50.6-  
195 years and age of asthma-onset of 23.6-years (Table 1). The commonest physical  
196 comorbidities ever reported (diagnostic definitions; Table E2) were rhinitis, GORD (gastro-  
197 oesophageal reflux disease) and obesity with high prevalence also of psychophysiologic  
198 (anxiety, depression, dysfunctional breathing and intermittent laryngeal dysfunction)  
199 comorbidity. Half the cohort (47.6%) had ever smoked, (current smokers = 5.6%). At  
200 enrolment, in the preceding 12 months, 43.6% of the cohort had required multiple ( $\geq 3$ )  
201 courses of rescue or increased maintenance OCS, 29.9% were on maintenance OCS (mean  
202 dose = 12.0 mg/day), and 29.0% had been hospitalised due to asthma. Furthermore,  
203 17.6% were on biological asthma therapies and 28.2% had ever had an Intensive Care asthma  
204 admission (Table 1). Mean ACQ6 (Asthma Control Questionnaire) was 2.5. Two-thirds  
205 (68.0%; 266/391) were atopic on SPT. Mean post-bronchodilator FEV1/FVC ratio was  
206 obstructive at 66.4%.

**207 Age of Onset and Sex Stratified Analyses****208 Demographic Characteristics**

209 The cohort was equally split between early (<18-years) and adult ( $\geq$ 18-years) onset disease  
210 (48.7% and 51.3% respectively). Age at study enrolment was significantly greater in males  
211 than females regardless of age of asthma-onset (Table 2) while females were more  
212 predominant both in early (71.3%) and adult-onset disease (59.5%). Females had  
213 proportionately more early (53.2%) than adult-onset disease (46.8%) but males had more  
214 adult (59.8%) than early-onset disease (40.2%). When stratified by age of onset and sex into  
215 four groups, 34.7% were early-onset female, 30.5% were adult-onset female, 20.8% were  
216 adult-onset male and 14% were early-onset male.

217 Males were younger at diagnosis than females among early-onset disease but older among  
218 adult-onset disease. Males were also significantly taller and heavier than female counterparts  
219 regardless of age of onset (Table E3). Age of onset and sex did not separately affect BMI  
220 (Body Mass Index) though their interaction led to significantly higher BMI in female than  
221 male adult-onset asthma (32.5 v 29.1). Female adult-onset subjects had highest obesity  
222 prevalence (Table 2). Obesity ( $\text{BMI} \geq 30$ ) was significantly affected by age of onset with a sex  
223 stratified interaction. While obesity prevalence was significantly lower in adult than early-  
224 onset asthmatic males it showed opposing patterns in females. Ever smoking history was  
225 significantly higher for both male and adult-onset status.

### 226 **Comorbidity Characteristics**

227 Rhinitis, GORD and sleep apnoea were equally common across the 4 groups and did not  
228 differ significantly by sex or age of asthma-onset (Table 3). Conversely, fungal sensitive  
229 disease (Allergic Bronchopulmonary Aspergillosis [ABPA]/Severe Asthma with Fungal  
230 Sensitivity [SAFS]) was significantly greater in males than females and early-onset than  
231 adult-onset disease. Clinically diagnosed non CF-bronchiectasis was significantly associated

232 with male sex regardless of age of asthma-onset. Radiological bronchiectasis or emphysema  
233 on HRCT scan did not show significant association to sex or age of onset (Table 4).

234 Several physical (salicylate [Non-Steroidal Anti-Inflammatory drug; NSAID] sensitivity,  
235 sulphite sensitivity, latex allergy) and psychophysiologic (depression, anxiety, dysfunctional  
236 breathing and intermittent laryngeal dysfunction) disorders were significantly associated with  
237 female sex regardless of age of asthma-onset. History of bariatric surgery was also  
238 significantly associated with female sex (overall prevalence; 1.65%, sex difference  $p =$   
239 0.007). Regardless of sex, significant age of onset associations were noted for eczema (more  
240 prevalent in early-onset asthma), COPD and eosinophilic granulomatosis with polyangiitis  
241 (EGPA) (both more prevalence in adult-onset asthma).

#### 242 **Objective Disease Phenotype Characteristics**

243 Atopic status and Total IgE were significantly influenced by age of onset, being greatest in  
244 early-onset groups with no sex differences (Table 4). Conversely, skin test positivity to  
245 aspergillus was significantly associated with male sex regardless of age of onset. Peripheral  
246 eosinophil counts at enrolment did not differ significantly by age or sex but FeNO was  
247 significantly higher in adult-onset groups regardless of sex.

248 Sex significantly influenced post-bronchodilator (BD) spirometry with significantly lower  
249 FEV1, FVC, and FEV1/FVC predicted values in males than females (Figure 1 and Table E4).

250 A significant interaction between sex and age of onset ( $p = 0.002$ ) was shown for both FEV1  
251 and FVC which were higher for adult-onset compared to early-onset males but no different  
252 in females (Figure 1; Table E4). Neither age or sex affected transfer factor (DLCO/KCO)  
253 measures at enrolment (Table 4). We found no differences in FEV1 between ever smokers  
254 and never smokers within each phenotype based on reported current, past or never smoking  
255 status. Thus, whilst smoking prevalence differed between phenotypes, the simple parameter

256 of ever smoking status did not significantly account for differences in FEV1 between these  
257 groups.(Figure 2; full data in Table E5).

### 258 **Disease-Related Questionnaire Characteristics**

259 ACQ6 was significantly affected by interaction of sex and age of onset with lowest (best)  
260 values in adult-onset males. HADS (Hospital Anxiety and Depression Score), Nijmegen  
261 score, Hull cough score and SNOT-22 score were significantly higher in females regardless  
262 of age of asthma-onset (Table 5).

### 263 **Treatment & Healthcare Utilisation Characteristics**

264 Maintenance OCS use was highest in male adult-onset disease reflecting a significant  
265 interaction between male sex and age of onset ( $p=0.014$ ); no such associations were observed  
266 in females (Table E6 and Table E7). Maintenance OCS use and dosage, number of rescue  
267 OCS courses and use of biological therapies did not differ by phenotype. Ever needing ICU  
268 admission and history of intubation for asthma was greater in early-onset disease ( $p=0.001$ )  
269 irrespective of sex but recent hospitalisations were equally prevalent across phenotypes  
270 (Table E6 and E7).

### 271 **Summary Of Age of onset/ Sex Phenotypes**

272 Summary characterization of the 4 age of onset/ sex phenotypes is displayed in Figure 3.

273

**274 DISCUSSION**

275 To our knowledge, this is the first description of a clinical cohort of difficult asthma patients  
276 using a simple phenotypic stratification based on their sex and age of asthma-onset. WATCH  
277 cohort participants showed high asthma morbidity and comorbidities, consistent with the  
278 concept of difficult asthma. The cohort had a higher proportion of females but equal  
279 proportions of early and adult-onset disease. Males developed asthma younger than females  
280 among the early-onset asthmatics but later within the adult-onset groups, suggesting biphasic,  
281 age-related associations for male difficult asthma. The identified phenotypes differed in  
282 respect of lung function, FeNO, atopic status, fungal sensitisation, smoking status, oral  
283 corticosteroid needs and both physical and psychophysiologic comorbidities. This highlights  
284 the heterogeneous nature of difficult asthma in relation to age of asthma-onset and/or sex.  
285 Such understanding may have important clinical implications.

**286 Differential associations of sex and age of onset with aspects of Difficult Asthma**

287 Our observations are consistent with female predominance reported in three out of four  
288 secondary care clusters in the Leicester study<sup>15</sup>, all five clusters in BTS Difficult Asthma  
289 Registry<sup>17</sup> and SARP<sup>16</sup> studies, and in three out of four clusters in UBIOPRED<sup>18</sup>. Using a  
290 different approach, we identified both early and adult-onset forms of male difficult asthma,  
291 with males disproportionately more likely to have adult-onset disease. Whether such patterns  
292 represent a distinctive feature of difficult compared to milder asthma is unclear. Of note,  
293 within SARP III, regardless of disease severity, childhood-onset asthma demonstrated male  
294 predominance<sup>28</sup>. Comparison to milder asthmatics in the Isle of Wight Whole Population  
295 Birth Cohort (IOWBC)<sup>29</sup> and also in our mild adult asthma study cohort (Table E8; online  
296 repository text) suggests that there may indeed be different associations of sex for mild and  
297 difficult asthma. Previous severe asthma cluster studies also reported varied age of onset/sex  
298 relationships. In the Leicester study, three secondary care clusters were childhood-onset/

299 female predominant and the one adult-onset cluster predominantly male<sup>15</sup>. Of the three  
300 Leicester primary care clusters, two were adult-onset female predominant while the  
301 childhood-onset cluster was male predominant. In SARP, three clusters were childhood and  
302 two adult-onset (all female predominant)<sup>16</sup>. In the BTS Registry four clusters were adult-  
303 onset (all female predominant)<sup>17</sup>. UBIOPRED reported two childhood and two adult-onset  
304 severe asthma clusters with male and female predominant groups in each<sup>18</sup>. Such variation  
305 may reflect both population and methodological/ classification differences.

306 In our study, males had impaired spirometry accompanied by a significant age-related  
307 interaction whereby early-onset males had lowest FEV<sub>1</sub> and FVC. Those findings in early-  
308 onset male difficult asthma may represent effects of longer disease duration, associated with  
309 more pronounced airway remodelling, fixed airflow limitation and consequent air  
310 trapping<sup>30,31</sup>. They also align with findings of persistently low lung function trajectories  
311 established in early life that track longitudinally<sup>32-35</sup>. However, our findings for males were  
312 not seen in female early-onset subjects whose disease duration was also long. Timing of  
313 disease onset, before or after pubertal growth spurt, and parallel/ subsequent environmental  
314 exposures might impact on lung function to account for such apparent sex differences. Our  
315 early-onset male asthmatics showed raised smoking prevalence, which may have contributed  
316 to their impaired adult lung function. While smoking uptake in early-onset male asthmatics is  
317 contrary to conventional expectation, it mirrors prior descriptions of smoking uptake in  
318 asthma patients in EGEA and IOWBC studies<sup>36,37</sup>. This, therefore, identifies an important  
319 focus for improved clinical management. Adult-onset males also had higher smoking  
320 prevalence and poorer lung function than female counterparts despite shortest disease  
321 duration. However, while adult-onset females had higher smoking prevalence than early-  
322 onset females, their lung function was not significantly worse, questioning how much  
323 smoking additionally influenced lung function differences between early and adult-onset

324 females. One limitation of our dataset is the lack of more detailed information on smoking  
325 duration and consumption levels (ie pack year history). Therefore our findings that ever  
326 smoking status did not add significantly to the effects of age of onset and sex upon lung  
327 function remain speculative and should be interpreted with caution. Unfortunately our dataset  
328 lacks the depth to assess causal association of smoking with observed lung function  
329 differences. Future assessment of these phenotypes should seek to assess this point using  
330 detailed smoking information to give clarity on the nature of that potential association.

331 Salicylate (NSAID) sensitive asthma is known to be more severe and we found that to be  
332 more prevalent in females, confirming existing understanding<sup>38</sup>. The absence of a positive  
333 formal drug challenge for diagnosis of this state is a potential limitation in our study that  
334 might raise concerns of overdiagnosis in our data. However patient reported symptoms in the  
335 presence of an appropriate clinical phenotype have been previously shown to align to high  
336 likelihood of positive aspirin challenge<sup>38</sup>. While generally regarded as associated with  
337 adult-onset asthma, we also demonstrated it among early-onset females. That aligns with  
338 recent reports of early-onset forms of this phenotype that merit awareness<sup>39</sup>. Our findings  
339 associating sulphite sensitivity with females are less well recognised<sup>40</sup>. Fungal sensitivity is  
340 also a hallmark of more severe asthma and we found that ABPA/SAFS and aspergillus were  
341 commonest in males<sup>41</sup>. That is also not well documented in the literature where either no sex,  
342 or female predominance have generally been reported<sup>42,43</sup>. Highest prevalence occurred in  
343 early-onset male asthma contrasting with findings of later-onset fungal disease in other  
344 populations<sup>43</sup>. Much interest has recently focused on associations of obesity with more  
345 problematic asthma<sup>14</sup>. In this study, we confirmed high prevalence of obesity in difficult  
346 asthma, which was significantly influenced by interaction of sex with age of asthma-onset.  
347 Our findings of greater obesity prevalence in females with difficult asthma are consistent  
348 with other severe asthma cohorts and likely multifactorial in origin<sup>15-18,28</sup>. Opposing obesity

349 prevalence for adult-onset asthmatic males and females in WATCH highlight potential sex-  
350 stratified mechanistic differences for adult-onset difficult asthma<sup>44</sup>.

351 Psychophysiologic comorbidity was common in WATCH and showed female predominance,  
352 with depression and anxiety more common in females. A potentially complex interplay of  
353 chronic inflammation, sex hormones and psychosocial factors may influence such  
354 associations<sup>45</sup>. Dysfunctional breathing and intermittent laryngeal obstruction also showed a  
355 female predominance and high prevalence in our cohort, consistent with other studies<sup>46,47</sup>.

356 Psychophysiologic comorbidity has been frequently noted in adult-onset female severe  
357 asthma clusters, including cluster 2 in the BTS Registry<sup>17</sup>, cluster T4 in U-BIOPRED<sup>18</sup> and  
358 cluster 2 in the Leicester secondary care cluster<sup>15</sup>. Such clusters manifest disproportionately  
359 high symptom expression for degree of airway pathophysiology, suggesting a  
360 multicomponent framework for their breathlessness<sup>15,17,18</sup>. In WATCH, whilst confirming  
361 high psychophysiologic comorbidity among a similar adult-onset female group, highest  
362 prevalence actually occurred in early-onset females who also had strong pathophysiological  
363 disease signals. Individuals with longer asthma duration might plausibly manifest  
364 psychophysiologic comorbidity as a consequence of detriment from their prolonged airways  
365 disease though seemingly more so in females.

### 366 **Relevance and Implications of WATCH Phenotypes**

367 Presentation to secondary or tertiary care with difficult asthma is often decades after disease  
368 onset. Greater awareness of asthmatics at risk for more difficult disease outcomes should  
369 support earlier identification and more targeted treatment, potentially alleviating decades of  
370 suffering from poorly controlled disease. In that regard, comparing to preceding analyses  
371 using unbiased data modelling, WATCH early-onset male difficult asthmatics are not clearly  
372 distinguished in the Leicester<sup>15</sup>, SARP<sup>16</sup>, BTS Registry<sup>17</sup> or UBIOPRED<sup>18</sup> studies but share

373 features with childhood-onset male severe asthmatics in SARP III<sup>28</sup>. Significant airflow  
374 limitation shows them worthy of attention and likely to benefit from focused efforts to  
375 prevent smoking uptake. WATCH adult-onset female difficult asthmatics shared features  
376 with previously described clusters including cluster T4 in UBIOPRED<sup>18</sup>, cluster 3 in SARP<sup>16</sup>  
377 and cluster 2 in the BTS Registry<sup>17</sup>. Early-onset female difficult asthma was the commonest  
378 grouping in WATCH and resembled aspects of cluster 1 in the BTS Registry<sup>17</sup>, clusters 1 and  
379 3 in the Leicester secondary care cohort<sup>15</sup> and clusters 1 and 2 in SARP<sup>16</sup>. It is therefore  
380 important to recognise that a fair proportion of difficult/severe asthma in adult females  
381 originates in early life. Consequently, it is worth considering if better recognition and  
382 management of childhood asthma in girls might reduce later disease morbidity.

383 The adult-onset male difficult asthmatic observed in WATCH is poorly acknowledged in  
384 clinical practice. Evolving into more difficult disease shortly after onset, as judged by the  
385 short disease duration in WATCH, they had the worst lung function, high smoking  
386 prevalence and high maintenance OCS use. Overlaps of asthma with COPD are now well  
387 recognised and it could be speculated that this group represents such disease duality<sup>48</sup>, yet  
388 while diagnosed COPD was significantly associated with older age of onset it was not  
389 significantly associated with male sex in WATCH. Of note, adult-onset males did not show  
390 significantly greater radiological emphysema or impairment of gas transfers. Furthermore,  
391 despite their smoking history they had highest FeNO levels (a biomarker that is normally  
392 suppressed by cigarette smoke) and trends for highest peripheral eosinophils, both signs of  
393 T2-high inflammation. Given their other disease features their low ACQ scores may actually  
394 represent relative symptom under-perception which is previously reported in males<sup>49,50</sup>.

395 Neither SARP<sup>17</sup>, ECRHS II<sup>51</sup> nor UBIOPRED<sup>18</sup> studies clearly identified this group.  
396 However, a similar group was seen as cluster 4 in the Leicester secondary care cluster that  
397 showed male predominance, raised FeNO, higher eosinophilia, lower lung function and oral

398 corticosteroid dependency<sup>15</sup>. Though differing in some characteristics, male adult-onset  
399 troublesome asthma is noted as phenotype F in the EGEA2 Study<sup>51</sup>, cluster 2 in the Seinäjoki  
400 Adult Asthma Study (SAAS)<sup>13</sup> and cluster 6 in the Taiwanese Adult Asthma Cohorts study<sup>52</sup>.  
401 Cluster 4 in the BTS Registry, whilst not male predominant, showed highest male prevalence  
402 in that study alongside adult-onset, higher smoking and impaired lung function<sup>17</sup>.

### 403 **Strengths and limitations**

404 An important strength of this study is that the WATCH cohort represents an extensively  
405 characterized real-life difficult asthma clinic population. Patients are drawn from many  
406 locations across a large geographical area across Southern England, effectively making the  
407 study multi-site. The WATCH participants showed levels of morbidity comparable to  
408 previous Severe Asthma cohorts<sup>15-18</sup>. However, WATCH specifically focuses on the more  
409 common clinical entity of difficult rather than severe asthma and therefore potentially has  
410 wider clinical applicability. As with any study, there are limitations. WATCH is a pragmatic  
411 study aligned to clinic attendance and therefore there is some missing data though that is not  
412 substantial. Because entry into the study is entirely voluntary, we cannot exclude that some  
413 phenotypes were more likely to enrol. Age of onset data is collected at enrolment into  
414 WATCH and is, therefore, prone to an element of recall bias, a common issue in all severe or  
415 difficult asthma studies. Our definition of early-onset is <18yrs, merging childhood and  
416 adolescence, which has the potential to oversimplify differential influence of sex on asthma  
417 in earlier life. Nevertheless, this age cut off results in a clear definition of adult-onset disease  
418 mirroring paediatric/ adult respiratory care transition and is, therefore, clinically applicable.  
419 The use of clinical diagnoses to define comorbidities might also be criticised but reflects real-  
420 life clinical practice. Finally, our study population is largely Caucasian, representative of the  
421 locality. Our findings need replication in other populations to assess wider applicability.

**422 Conclusion**

423 This study depicts a diverse nature to difficult asthma as a multicomponent problem with  
424 numerous comorbidities. Assessment based on age of disease onset and sex shows that these  
425 factors, and sometimes their interaction, are relevant to different clinical manifestations of  
426 difficult asthma. That perspective highlights patient groups that are currently poorly  
427 recognised in clinical practice. In particular, early-onset female and adult-onset male difficult  
428 asthma phenotypes need recognition to improve their outcomes and future research should  
429 focus further on the mechanisms underlying their clinical symptoms.

430

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Journal Pre-proof

632 **Table 1 Summary Characteristics of the Overall WATCH Cohort at Enrolment.**

	<i>Valid Cases Data Available</i>	<i>Mean (SD)</i>	<i>% (N)</i>
<i>Demographics</i>			
<b>Female</b>	501 (100%)		65.3% (327)
<b>Age at Study Enrolment (years)</b>	501 (100%)	50.6 (15.8)	
<b>Age at Asthma Diagnosis</b>	479 (95.6%)	23.6 (20.5)	
<b>BMI (kg/m<sup>2</sup>)</b>	495 (98.8%)	30.9 (7.23)	
<b>Obese (BMI &gt; 30 kg/m<sup>2</sup>)</b>	495 (98.8%)		48.3% (239)
<b>Current or Ex Smokers</b>	500 (99.8%)		47.6% (238)
<i>Co-Morbidities</i>			
<b>Rhinitis</b>	446 (89.0%)		67.5% (301)
<b>Eczema</b>	495 (98.8%)		26.1% (129)
<b>ABPA or SAFS<sup>†</sup></b>	493 (98.4%)		6.9% (34)
<b>Bronchiectasis</b>	495 (98.8%)		14.1% (70)
<b>GORD</b>	486 (97.0%)		64.8% (315)
<b>Depression</b>	454 (90.6%)		36.8% (167)
<b>Anxiety</b>	451 (90.0%)		32.8% (148)
<b>Dysfunctional Breathing</b>	476 (95.0%)		48.7% (232)
<b>Intermittent Laryngeal Dysfunction</b>	447 (89.2%)		14.5% (65)
<b>Sulphite Sensitivity*</b>	493 (98.4%)		7.7% (38)
<b>Salicylate Sensitivity*</b>	495 (98.8%)		25.1% (124)
<b>EGPA</b>			1.0% (5)
<b>Sleep Apnoea</b>			7.2% (35)
<i>Healthcare Utilisation</i>			
<b>≥1 Asthma Related ICU Visits ever</b>	500 (99.8%)		28.2% (141)
<b>≥1 Asthma Hospital Admission (last 12 months)</b>	497 (99.2%)		29.0% (144)
<b>≥3 Rescue Oral Corticosteroids (last 12 months)</b>	448 (89.4%)		43.6% (240)

<b>Treatment</b>			
<b>Inhaled Corticosteroid Dose (BDP equivalent; ucg)</b>	412 (82.2%)	2593.41 (985.27)	
<b>Maintenance Oral Corticosteroid</b>	475 (94.8%)		30.5% (145)
<b>OCS Dose (for those on maintenance oral corticosteroid; mg)</b>	140 (27.9%)	12.03 (8.49)	
<b>Biologic Therapy</b>	495 (98.8%)		17.6% (87)
<b>Blood Test Results</b>			
<b>Eosinophil Count (10<sup>9</sup> cells/L)</b>	455 (90.8%)	0.3 (0.33)	
<b>Total IgE (iu/mL)</b>	384 (76.6%)	378 (1336)	
<b>Lung Function Test Results</b>			
<b>FeNO50 (ppb)</b>	390 (77.8%)	30.8 (34.43)	
<b>Post BD FEV1 (%)</b>	341 (68.1%)	75.6 (22.79)	
<b>Post BD FEV1/FVC (ratio)</b>	341 (68.1%)	66.4 (14.62)	
<b>DLCO (%)</b>	198 (39.5%)	99.7 (17.97)	
<b>Skin Prick Tests</b>			
<b>Positive to any Aeroallergen (≥3mm)</b>	391 (78.0%)		68.0% (266/391)
<b>Positive to Aspergillus (≥3mm)</b>	355 (70.9%)		15.8% (56)
<b>Questionnaires</b>			
<b>ACQ6 Score (good control &lt;1.5)</b>	467 (93.2%)	2.5 (1.36)	
<b>Epworth Score (normal &lt;11)</b>	424 (84.6%)	8.3 (5.5)	
<b>HADS A Score (normal &lt;8)</b>	418 (83.4%)	7.06 (4.78)	
<b>HADS D Score (normal &lt;8)</b>	418 (83.4%)	5.41 (4.39)	
<b>Hull Cough Score (normal &lt;14)</b>	378 (75.4%)	26.2 (15.68)	
<b>Nijmegen Score (normal &lt;23)</b>	373 (74.5%)	22.2 (12.4)	
<b>SNOT22 Score (normal &lt;8)</b>	324 (64.7%)	35.2 (21.35)	

633 Table 1. Quantitative variables expressed as mean (SD) and Categorical variables as n(%). Abbreviations: BMI = Body Mass  
634 Index, ABPA = Allergic Bronchopulmonary Aspergillosis, SAFS = Severe Asthma Fungal Sensitisation, GORD = Gastro-  
635 Oesophageal Reflux Disease, EGPA = Eosinophilic Granulomatosis with Polyangiitis, ICU = Intensive Care Unit, BDP =  
636 Beclomethasone, OCS = Oral Corticosteroid, IgE = Immunoglobulin E, FENO50 = Fractional Exhaled Nitric Oxide at Expiratory  
637 Rate of 50ml/s in parts per billion (ppb), Post BD = post bronchodilator, FEV1 = Forced Expiratory Volume in 1 second, FVC =  
638 Forced Vital Capacity, DLCO = Diffusing Capacity of Lung for Carbon Monoxide, ACQ6 = 6 Item Asthma Control  
639 Questionnaire, HADS = Hospital Anxiety (A) and Depression (D) Score, SNOT22 = SinoNasal Outcome Test 22. \* Determined  
640 clinically based on compatible history of reaction on exposure and consistent clinical phenotypes. † Determined clinically  
641 using evidence of relevant serological and radiological information guided by conventional clinical diagnostic criteria (eg  
642 reference 41).

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644 **Table 2 Demographic Characteristics of Age of onset/ Sex Phenotypes.**

	Male Early Onset (n=70)	Female Early Onset (n=174)	Male Adult Onset (n=104)	Female Adult Onset (n=153)	Adult Onset Effect		Male Effect	
<b>*Age (at Enrolment) Mean (SD)</b>	49.97 (15.45)	40.51 (14.85)	61.92 (11.25)	54.77 (12.26)	13.48 (11.09 ; 15.86)	$p<0.001$	8.18 (5.68 ; 10.68)	$p<0.001$
<i>Missing cases</i>	0	0	0	0				
<b>*Age at Diagnosis Mean (SD)</b>	4.27 (4.74)	6.66 (5.98)	46.98 (14.16)	35.39 (13.49)	Interaction effect $p<0.001$ (see below)			
<i>Missing cases</i>	6	7	6	3				
<b>*BMI (mean SD)</b>	30.11 (5.86)	30.87 (8.22)	29.01 (5.49)	32.50 (7.42)	Interaction effect $p=0.047$ (see below)			
<i>Missing cases</i>	2	2	2	0				
<b>†Obese (BMI&gt;30) %</b>	48.53%	49.42%	31.37%	58.17%	Interaction effect $p=0.006$ (see below)			
<i>Missing Cases</i>	2	2	2	0				
<b>†Never Smokers %</b>	42.86%	65.52%	37.50%	51.97%	1.56 (1.09 ; 2.25)	$p=0.015$	2.1 (1.44 ; 3.08)	$p<0.001$
<i>Missing cases</i>	0	0	0	1				
					<i>Effect of Late onset for Male</i>		<i>Effect of Late Onset for Female</i>	
				<b>*Age at Diagnosis</b>	42.71 (39.12 ; 46.31)	$p<0.001$	28.73 (26.47 ; 30.99)	$p<0.001$
				<b>*BMI</b>	-1.1 (-2.83 ; 0.63)	<i>ns</i>	1.63 (-0.08 ; 3.34)	<i>ns</i>
				<b>†Obese</b>	0.48 (0.26 ; 0.91)	$p=0.024$	1.42 (0.92 ; 2.21)	<i>ns</i>

645 Table 2. \*Quantitative variables expressed as Mean (SD). Early-onset <18-years; Adult-onset ≥18-years. Adult Onset and Male Effects explored by two way ANOVA and expressed as difference  
646 of means (95% confidence intervals). For Age at Diagnosis and BMI, where a significant interaction result was found, a one way ANOVA of late onset for each gender is performed. †Categorical  
647 variables expressed as percentages within each group. Adult Onset and Male Effects explored by logistic regression models and expressed as an odds ratio (95% confidence intervals). For  
648 Obesity, where a significant interaction result was found, a logistic regression model of late onset for each sex is performed. Abbreviations: BMI = body mass index, *ns* = not significant.

649

650 Table 3 Common Comorbidities of Age of onset/ Sex Phenotypes.

	Male Early Onset (n=70)	Female Early Onset (n=174)	Male Adult Onset (n=104)	Female Adult Onset (n=153)	Adult Onset Effect		Male Effect	
<b>*Rhinitis</b>	69.84%	68.35%	67.37%	65.38%		<i>ns</i>		<i>ns</i>
Missing cases	7	16	9	23				
<b>*Eczema</b>	38.57%	39.41%	11.65%	15.13%	0.25 (0.16 ; 0.38) °	<i>p</i> <0.001		<i>ns</i>
Missing cases	0	4	1	1				
<b>*ABPA/SAFS</b>	14.49%	8.24%	6.73%	2.00%	0.33 (0.14 ; 0.69)	<i>p</i> =0.003	2.27 1.11 ; 4.68)	<i>p</i> =0.025
Missing cases	1	4	0	3				
<b>*Bronchiectasis</b>	23.19%	8.19%	19.23%	13.25%		<i>ns</i>	2.18 (1.30 ; 3.66)	<i>p</i> =0.003
Missing Cases	1	3	0	2				
<b>*GORD</b>	63.77%	63.47%	67.37%	67.79%		<i>ns</i>		<i>ns</i>
Missing cases	1	7	3	4				
<b>*COPD</b>	10.14%	3.49%	17.31%	12.58%	2.78 (1.47 ; 5.59)	<i>p</i> <0.001		<i>ns</i>
Missing cases	1	2	0	4				
<b>*Depression</b>	27.87%	47.44%	24.74%	37.14%		<i>ns</i>	0.49 (0.32 ; 0.75)	<i>p</i> =0.001
Missing cases	9	18	7	13				
<b>*Anxiety</b>	28.57%	40.38%	21.88%	33.82%		<i>ns</i>	0.57 (0.36 ; 0.87)	<i>p</i> =0.010
Missing cases	7	18	8	17				
<b>*Dys. Breathing</b>	41.79%	59.01%	40.00%	46.62%		<i>ns</i>	0.63 (0.43 ; 0.93)	<i>p</i> =0.019
Missing cases	3	13	4	5				
<b>*ILO</b>	9.52%	17.65%	8.99%	16.90%		<i>ns</i>	0.49 (0.25 ; 0.89) ‡	<i>p</i> =0.018
Missing cases	7	21	15	11				
<b>*Sulphite Sensitivity</b>	2.90%	12.43%	4.85%	6.58%		<i>ns</i>	0.40 (0.16 ; 0.87) ‡	<i>p</i> =0.020
Missing cases	1	5	1	1				
<b>*Salicylate Sensitivity</b>	10.29%	28.90%	13.59%	35.10%		<i>ns</i>	0.30 (0.18 ; 0.49) ‡	<i>p</i> <0.001
Missing cases	2	1	1	2				
<b>*EGPA</b>	0	0	2.88%	1.33%		n/a†		<i>ns</i>
Missing cases	1	4	0	3				
<b>*Sleep Apnoea</b>	10.61%	6.43%	8.82%	5.33%		<i>ns</i>		<i>ns</i>
Missing cases	4	3	2	3				

651 *Table 3 \*Categorical variables expressed as percentages within each group. Early-onset <18-years; Adult-onset ≥18-years. Adult Onset and Male Effects explored by logistic regression models*  
652 *and expressed as an odds ratio (95% confidence intervals). Abbreviations: ABPA = Allergic Bronchopulmonary Aspergillosis, SAFS = Severe Asthma Fungal Sensitisation, GORD = Gastro-*  
653 *Oesophageal Reflux Disease, COPD = Chronic Obstructive Pulmonary Disease, Dys. = Dysfunctional, ILO – Intermittent Laryngeal Dysfunction, EGPA = Eosinophilic Granulomatosis with*  
654 *Polyangiitis. † unable to express odds ratio as frequency for early-onset is 0, ‡non adjusted estimated odds ratio because the one-factor model was best than two-factors model (by stepwise*  
655 *selection), but very similar as adjusted estimated odds ratio, ns = not significant.*

656

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657 **Table 4 Objective characterisation test results for Age of onset/ Sex Phenotypes.**

	Male Early Onset (n=70)	Female Early Onset (n=174)	Male Adult Onset (n=104)	Female Adult Onset (n=153)	Adult Onset Effect		Male Effect	
<b>*Blood Eosinophil (10<sup>9</sup> cells/L)</b>	0.25 (0.21)	0.26 (0.38)	0.31 (0.38)	0.24 (0.28)		<i>ns</i>		<i>ns</i>
<i>Missing cases</i>	9	17	6	15				
<b>*Total Serum IgE (iu/mL)</b>	638 (988)	485 (2073)	74 (665)	141 (266)	-291.27 (-557.38 ; -25.16) ‡	<i>p=0.032</i>		<i>ns</i>
<i>Missing cases</i>	19	38	24	36				
<b>*FeNO50 (ppb)</b>	22.91 (24.64)	25.40 (28.77)	41.49 (41.44)	32.56 (36.49)	11.15 (4.33 ; 17.96)	<i>p=0.001</i>		<i>ns</i>
<i>Missing cases</i>	16	44	17	34				
<b>*DLCO (%pred)</b>	86.31 (19.04)	91.01 (15.33)	85.74 (22.49)	87.17 (18.28)		<i>ns</i>		<i>ns</i>
<i>Missing cases</i>	44	102	69	88				
<b>*KCO (%pred)</b>	105 (18)	99 (15)	101 (22)	97 (18)		<i>ns</i>		<i>ns</i>
<i>Missing cases</i>	44	102	69	88				
<b>†Positive SPT</b>	83.64%	76.92%	60.76%	54.39%	0.36 (0.23 ; 0.55) ‡	<i>p&lt;0.001</i>		<i>ns</i>
<i>Missing cases</i>	15	31	25	39				
<b>†Aspergillus + SPT</b>	28.85%	14.17%	18.67%	8.91%		<i>ns</i>	2.41 (1.34 ; 4.37)	<i>p=0.004</i>
<i>Missing cases</i>	18	47	29	52				
<b>HRCT Bronchiectasis</b>	26.83%	24.1%	22.86%	22.78%		<i>ns</i>		<i>ns</i>
<i>Missing cases</i>	29	91	34	74				
<b>HRCT Emphysema</b>	2.33%	7.32%	8.7%	13.58%		<i>ns</i>		<i>ns</i>
<i>Missing cases</i>	27	92	35	72				

658 Table 4 \*Quantitative variables expressed as Mean (SD). Early-onset <18-years; Adult-onset ≥18-years. Adult Onset and Male Effects explored by two way ANOVA and expressed as difference  
659 of means (95% confidence intervals). For PostBD FEV1 %pred, where a significant interaction result was found, a one way ANOVA of late onset for each sex is performed. †Categorical variables  
660 expressed as percentages within each group. Adult Onset and Male Effects explored by logistic regression models and expressed as an odds ratio (95% confidence intervals). Abbreviations: IgE  
661 = Immunoglobulin E, FENO50 = Fractional Exhaled Nitric Oxide at Expiratory Rate of 50ml/s in parts per billion (ppb), DLCO = Diffusing Capacity of Lung for Carbon Monoxide, KCO = Diffusing

662 *Capacity of Lung for Carbon Monoxide corrected for alveolar volume, SPT = skin prick test (to standard panel of aeroallergens), HRCT = High Resolution CT Chest. ‡ non adjusted estimated odds*  
663 *ratio because the one-factor model was best than two-factors model (by stepwise selection), but very similar as adjusted estimated odds ratio. ns = not significant.*

664

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665 **Table 5 Self Report Questionnaire Scores for Age of onset/ Sex Phenotypes.**

	Male Early Onset (n=70)	Female Early Onset (n=174)	Male Adult Onset (n=104)	Female Adult Onset (n=153)	Adult Onset Effect		Male Effect		
<b>*ACQ6</b>	2.59 (1.34)	2.59 (1.29)	2.03 (1.36)	2.68 (1.38)	<i>Interaction effect p=0.014 (see below)</i>				
<i>Missing cases</i>	3	9	10	12					
<b>*Epworth</b>	9.05 (5.56)	8.44 (5.31)	7.42 (5.35)	8.49 (5.78)	<i>ns</i>			<i>ns</i>	
<i>Missing cases</i>	12	25	14	26					
<b>*HADS A</b>	6.55 (4.86)	7.59 (4.77)	5.52 (4.10)	7.77 (4.92)	<i>ns</i>		-1.74 (-2.68 ; -0.80) †	<i>p&lt;0.001</i>	
<i>Missing cases</i>	10	28	15	23					
<b>*HADS D</b>	5.34 (3.93)	5.06 (4.46)	5.10 (4.25)	6.05 (4.51)	<i>ns</i>			<i>ns</i>	
<i>Missing Cases</i>	11	29	15	20					
<b>*HADS Total</b>	11.83 (7.85)	12.64 (8.48)	10.62 (7.90)	13.84 (8.50)	<i>ns</i>		-2.11 (-3.77 ; -0.45) †	<i>p=0.013</i>	
<i>Missing cases</i>	12	31	15	25					
<b>*Hull Cough</b>	21.80 (12.48)	27.26 (15.64)	21.71 (14.84)	29.92 (16.57)	<i>ns</i>		-6.78 (-10.04 ; -3.53) †	<i>p&lt;0.001</i>	
<i>Missing cases</i>	14	45	29	35					
<b>*Nijmegen</b>	20.76 (11.75)	23.76 (11.85)	17.91 (12.98)	24.08 (12.29)	<i>ns</i>		-4.82 (-7.41 ; -2.23) †	<i>p&lt;0.001</i>	
<i>Missing cases</i>	16	38	26	48					
<b>*SNOT 22</b>	30.60 (19.98)	37.56 (21.82)	30.21 (18.61)	37.71 (22.47)	<i>ns</i>		-7.25 (-12.12 ; -2.39) †	<i>p=0.003</i>	
<i>Missing cases</i>	23	50	42	62					
					<i>Effect of Late onset for Male</i>		<i>Effect of Late Onset for Female</i>		
					<b>ACQ6</b>	-0.56 (-0.98 ; -0.14)	<i>p=0.009</i>	0.09 (-0.21 ; 0.39)	<i>ns</i>

666 Table 5 \*Quantitative variables expressed as Mean (SD). Early-onset <18-years; Adult-onset ≥18-years. Adult Onset and Male Effects explored by two way ANOVA and expressed as difference  
667 of means (95% confidence intervals). For ACQ6, where a significant interaction result was found, a one way ANOVA of late onset for each sex is performed. Abbreviations: ACQ = Asthma  
668 Control Questionnaire, HADS = Hospital Anxiety and Depression Score, A = Anxiety, D = Depression, SNOT = SinoNasal Outcome Test, †non adjusted estimated odds ratio because the one-factor  
669 model was best than two-factors model (by stepwise selection), but very similar as adjusted estimated odds ratio. *ns* = not significant

670 **FIGURE LEGENDS**

671

672 **Figure 1 A-D: Post Bronchodilator Lung Function Characteristics for Age of Onset/Sex Phenotypes**

673

674 *Figure 1 Post Bronchodilator Spirometry Values for each phenotype: Early-onset <18-years; Adult-onset $\geq$ 18-years. A = Post BD FEV1 %*  
675 *predicted, B = Post BD FVC % predicted, C = Post BD FEV1/FVC Ratio, D = Post BD FEF 25-75 % predicted. An interaction effect by two*  
676 *way ANOVA is seen in Post BD FEV1 % predicted \*\* $p=0.002$ . Post hoc one way ANOVA demonstrates an effect of age of diagnosis in males*  
677 *but not females. An interaction effect by two ANOVA is seen in Post BD FVC % predicted \*\* $p=0.002$ . Post hoc one way ANOVA demonstrates*  
678 *an effect of age of diagnosis in males but not females. \*\*\*\*In Post BD FEV1/FVC Ratio and Post BD FEF 25-75 % predicted there is a*  
679 *significant difference between male and female,  $p<0.001$  and  $p<0.001$  respectively. A full breakdown of these statistics is available in Table E4*  
680 *online repository text.*

681

682 **Figure 2 Post Bronchodilator FEV1% predicted values for each phenotype stratified by smoking history**

683

684 *Figure 2 Post Bronchodilator FEV1% predicted values for each phenotype stratified by smoking history, ns = not significant, Early-onset <18-*  
685 *years; Adult-onset $\geq$ 18-years*

686

687

688 **Figure 3 Summary Characteristics of Difficult Asthma Age of Onset/Sex Phenotypes in the WATCH Cohort.**

689

690 *Figure 3 Summary of the four phenotypes in the WATCH Cohort of Difficult Asthma. The size of each quadrant is proportional to their*  
691 *prevalence within the WATCH cohort, Early-onset <18-years; Adult-onset $\geq$ 18-years*

1 **New Perspectives on Difficult Asthma; Sex and Age of Asthma-Onset Based**  
2 **Phenotypes - Supplementary Data**

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23 **Age of Onset vs Age of Diagnosis**

24 Age of onset of symptoms and age of diagnosis are both self-reported variables. These were  
25 reported to be identical in 267 of 382 cases (69.9%) and thus highly correlated ( $r_s = 0.932$ ,  $p < 0.001$ ).

26 **Comparison of WATCH and Non WATCH patients.**

27 In January 2018, the UHS Difficult Asthma Clinic was responsible for 980 patients. From this list 598  
28 patients were not participating in the WATCH Study at that time. 64 patients (6.5%) did not meet  
29 Step 4/5 criteria. 90 patients (16.9% of eligible patients) went on to join the WATCH study. From the  
30 remaining 444 patients, we collected data on age and gender from their demographic details and  
31 smoking status and treatment details (maintenance OCS and biologics use) from clinic letters.  
32 Complete data was only available for 408 patients (represented below). We compared these  
33 patients with all WATCH patients enrolled up until Mar 2019.

34 **Table E1: Comparison of WATCH and Non-WATCH Patient Characteristics**

	<b>WATCH (472)</b>	<b>Not WATCH (408)</b>	<b>p value</b>
<b>Age at enrolment median (IQR)</b>	52.00 (26)	53.00 (22)	$p < 0.05$
<b>Gender (% female)</b>	65.0%	66.2%	ns
<b>Smoking Status (% never)</b>	52.1%	51.7%	ns
<b>Maintenance OCS (%)</b>	30.1%	20.1%	$p < 0.001$
<b>Biologics (%)</b>	16.9%	15.2%	ns

35 *Table E1 Notes: Includes only those patients with complete data for all variables. Continuous variables expressed as Median*  
36 *(IQR) and compared across groups by Mann Whitney U test. Categorical variables expressed as percentages and compared*  
37 *across groups by Chi Square. OCS = oral corticosteroids. ns = not significant*

38

39 **Table E2: Diagnostic Criteria Used in Clinical Practice for Comorbidities**

Rhinitis	Physician clinical diagnosis
Eczema	Physician clinical diagnosis
ABPA or SABS <sup>†</sup>	Determined clinically using evidence of relevant serological and radiological information guided by conventional clinical diagnostic criteria (eg reference 41).
Bronchiectasis	Objective evidence (Radiological Features on any CT imaging of Chest including but not exclusive to HRCT)
COPD	Physician clinical diagnosis
GORD	Physician clinical diagnosis
Depression	Physician clinical diagnosis
Anxiety	Physician clinical diagnosis
Dysfunctional Breathing	Physician clinical diagnosis (supported by abnormal Nijmegen score)
Intermittent Laryngeal Dysfunction	Physician clinical diagnosis
Sulphite Sensitivity*	Physician clinical diagnosis based on compatible history of reaction on exposure and consistent clinical phenotypes
Salicylate Sensitivity*	Physician clinical diagnosis based on compatible history of reaction on exposure and consistent clinical phenotypes
EGPA	Physician clinical diagnosis based on objective evidence (serum results, radiological features, biopsy findings)
Sleep Apnoea	Physician clinical diagnosis (confirmed by sleep study)

40 *Table E2 Notes:*41 *COPD = Chronic Obstructive Pulmonary Disease, GORD = Gastro-oesophageal reflux disease, EGPA = Eosinophilic*42 *Granulomatosis with Polyangiitis*

43 **Table E3: Height Comparisons for Age of onset/Sex Phenotypes**

	Male Early Onset (n=70)	Female Early Onset (n=174)	Male Adult Onset (n=104)	Female Adult Onset (n=153)	Adult Onset Effect		Male Effect	
<b>*Height (cm)</b>	172.35 (7.6)	162.88 (6.58)	173.72 (6.49)	161.07 (6.99)	Interaction effect $p<0.001$ (see below)			
Missing cases	2	2	2	0				
					Effect of Late onset for Male		Effect of Late Onset for Female	
<b>*Height</b>					1.36 (-0.77 ; 3.5)	ns	-1.81 (-3.29 ; -0.34)	$p<0.05$

44 Table E3 Notes: \*Quantitative variables expressed as Mean (SD). Early-onset <18-years; Adult-onset  $\geq 18$ -years. Adult Onset and Male Effects explored by two way ANOVA and expressed as  
 45 difference of means (95% confidence intervals). ns = not significant

46 **Table E4: Post Bronchodilator (BD) Spirometry for Age of onset/Sex Phenotypes**

	Male Early Onset (n=70)	Female Early Onset (n=174)	Male Adult Onset (n=104)	Female Adult Onset (n=153)	Adult Onset Effect		Male Effect	
<b>*PostBD FEV1 (%pred)</b>	60.03 (19.92)	81.25 (21.10)	72.86 (20.84)	78.79 (23.61)	Interaction effect $p=0.002$ (see below)			
Missing Cases	20	65	33	42				
<b>*Post BD FEV1/FVC (%pred)</b>	58.18 (14.38)	71.78 (11.76)	60.54 (13.12)	68.42 (15.44)	ns		-10.42 (-13.49 ; -7.36)	$p<0.001$
Missing cases	20	65	33	42				
<b>Post BD FVC (%pred)</b>	81.49 (17.89)	91.96 (17.78)	93.2 (19.07)	90.84 (17.68)	Interaction effect $p=0.002$ (see below)			
Missing Cases	20	65	33	43				
					Effect of Late onset for Male		Effect of Late Onset for Female	
<b>Post BD FEV1 %pred</b>					12.83 (5.43 ; 20.24)	$p=0.001$		ns
<b>Post BD FVC %pred</b>					11.71 (4.98 ; 18.44)	$p<0.001$		ns

48 Table E4 Notes: \*Quantitative variables expressed as Mean (SD). Early-onset <18-years; Adult-onset ≥18-years. Adult Onset and Male Effects explored by two way ANOVA and expressed as  
 49 difference of means (95% confidence intervals). For PostBD FEV1 %pred, where a significant interaction result was found, a one way ANOVA of late onset for each sex is performed. ns = not  
 50 significant

51

52 **Table E5: Effect of Smoking History on \*PostBD FEV1 (%pred) on Phenotypes**

	Male Childhood Onset	Female Childhood Onset	Male Adult Onset	Female Adult Onset
<b>Never Smoker</b>	62.5 (21.2)	83.1 (21.9)	74.0 (18.8)	79.8 (20.1)
<i>Valid cases</i>	21	75	27	57
<b>Current/Ex Smoker</b>	58.2 (19.1)	77.3 (19.0)	72.2 (22.2)	76.8 (26.4)
<i>Valid cases</i>	29	34	44	53
	ns	ns	ns	ns

53 *Table E5 Notes: \*Quantitative variables expressed as Mean (SD). Early-onset <18-years; Adult-onset ≥18-years.*54 **Table E6: Healthcare Utilisation and Treatment Requirements for Age of onset/ Sex Phenotypes.**

	Male Childhood Onset (n=70)	Female Childhood Onset (n=174)	Male Adult Onset (n=104)	Female Adult Onset (n=153)	Adult Onset Effect		Male Effect	
<b>*Mean ICS Dose (BDP equivalent; ucg)</b>	2578.84 (916.93)	2573.12 (967.58)	2449.17 (970.46)	2531.12 (1028.86)	-205.6 (-395.51 ; -15.69) °	p=0.034		ns
<i>Missing cases</i>	15	37	14	23				
<b>#Maintenance OCS</b>	21.88%	29.09%	37.62%	31.03%		ns		ns
	6	9	3	8				
<b>*Mean Maintenance OCS Dose (mg)</b>	13.64 (9.03)	12.71 (8.88)	10.7 (6.62)	11.94 (9.55)		ns		ns
	56	127	66	112				
<b>#Biologic Therapy</b>	18.84%	20.35%	18.27%	13.33%		ns		ns
<i>Missing cases</i>	1	2	0	3				
<b>§&gt;1 ICU Visit ever</b>	35.71%	32.76%	26.92%	20.26%	0.49 (0.32 ; 0.75)	p=0.001		ns
<i>Missing Cases</i>	0	1	0	0				
<b>§&gt;1 Hospitalisations</b>	24.29%	31.03%	28.85%	30.72%		ns		ns

Missing cases	0	3	0	1			
<sup>§</sup> ≥3 OCS Courses	41.43%	45.98%	51.92%	50.32%			
Missing cases	9	20	12	12		ns	ns

55 Table E6 Notes: \*Quantitative variables expressed as Mean (SD). #Categorical variables expressed as percentages within each group. Early-onset <18-years; Adult-onset ≥18-years. Adult Onset  
 56 and Male Effects explored by logistic regression models and expressed as an odds ratio (95% confidence intervals). For Maintenance OCS, where a significant interaction result was found, a  
 57 logistic regression model of late onset for each sex is performed. <sup>§</sup>Count variables expressed as percentages of summary of post hoc defined categories – a full breakdown of each count is  
 58 available in Table E6. Adult Onset and Male Effects explored by negative binomial models and expressed as rate ratios (95% confidence intervals). Abbreviations: ICS = inhaled corticosteroid,  
 59 BDP = Beclomethasone, OCS = oral corticosteroid, ICU = intensive care unit, ° non adjusted estimated odds ratio because the one-factor model was best than two-factors model (by stepwise  
 60 selection), but very similar as adjusted estimated odds ratio. ns = not significant.

61 **Table E7: Detailed Healthcare Utilisation/ Steroid Data for Age of onset/Sex Phenotypes**

		Male Early Onset (n=70)	Female Early Onset (n=174)	Male Adult Onset (n=104)	Female Adult Onset (n=153)
ICU Visit Count	0	45 (64.29%)	116 (67.05%)	76 (73.08%)	122 (79.74%)
	1	12 (17.14%)	23 (13.29%)	19 (18.27%)	11 (7.19%)
	2 - 35	13 (18.57%)	34 (19.65%)	9 (8.65%)	20 (13.07%)
	Missing	0	1	0	0
Hospitalisations	0	53 (75.71%)	120 (70.18%)	74 (71.15%)	106 (69.74%)
	1	10 (14.29%)	19 (11.11%)	16 (15.38%)	18 (11.84%)
	2 – 20	7 (10%)	32 (18.71%)	14 (13.46%)	28 (18.42%)
	Missing	0	3	0	1
OCS Courses	0	13 (21.31%)	28 (18.18%)	21 (22.83%)	25 (17.73%)
	1	9 (14.75%)	28 (18.18%)	10 (10.87%)	23 (16.31%)
	2	10 (16.39%)	18 (11.69%)	7 (7.61%)	16 (11.35%)
	3	9 (14.75%)	17 (11.04%)	18 (19.57%)	22 (15.6%)
	4 – 20	20 (32.79%)	63 (40.91%)	36 (39.13%)	55 (39.01%)
	Missing	9	20	12	12

62 Table E7 Notes: Count variables expressed as percentages for each category. Early-onset <18-years; Adult-onset ≥18-years.

### 63 Mild Asthma Cohorts

64 The Isle of Wight Whole Population Birth Cohort (IOWBC) (n=1456) was established in 1989 to  
65 prospectively study the natural history of asthma and allergy through the life course. Follow-up at  
66 the David Hide Asthma and Allergy Research Centre, Isle of Wight has been conducted at regular  
67 intervals in the 1st 26-years (birth, 1, 2, 4, 10, 18 and 26-years) enabling thorough characterisation of  
68 the cohort utilising questionnaires, lung function testing, bronchial challenge testing, skin prick  
69 testing and blood testing. Now aged 30-years the cohort is estimated to include around 130 current  
70 mild asthmatics (BTS Step 1-3). The EOSA (Epigenetics of Severe Asthma) mild asthma cohort  
71 comprises 69 participants with mild asthma (BTS Step 1 or 2). These were drawn from either the  
72 IOWBC that has been followed to age 26-years to date or from clinics/ community recruitment on  
73 the Isle of Wight, UK.

74 To explore the unexpected finding of disproportionately less male early-onset difficult asthma we  
75 also assessed the sex distribution among mild asthmatics over the 1st 18-years of life in our IOWBC.  
76 Findings are shown in Table E6. Among 359 IOWBC subjects with early-onset mild asthma present at  
77 any point in the 1st 18-years there was an equal sex distribution while for IOWBC subjects with  
78 current mild asthma at 18-years there was moderate (55.4%) female predominance. The IOWBC has  
79 only been followed to 26-years precluding further assessment of relationships of sex to adult-onset  
80 asthma. We therefore also compared WATCH findings with a further small cohort (EOSA) of mild  
81 asthmatic volunteers (n=69) that included older subjects. There we found that a similar distribution  
82 of age of onset pertained in mild females as for the WATCH cohort. However contrasting with  
83 WATCH findings for difficult asthma, in that study  $\frac{3}{4}$  of males with mild asthma had early-onset  
84 disease. Such observations should not be over-interpreted but suggest possible differential  
85 relationships of male sex with age of disease onset for mild and difficult asthma, which warrant  
86 future research focus.

87

88 **Table E8: Age of onset and Sex Associations for Difficult and Mild Asthma Cohorts**

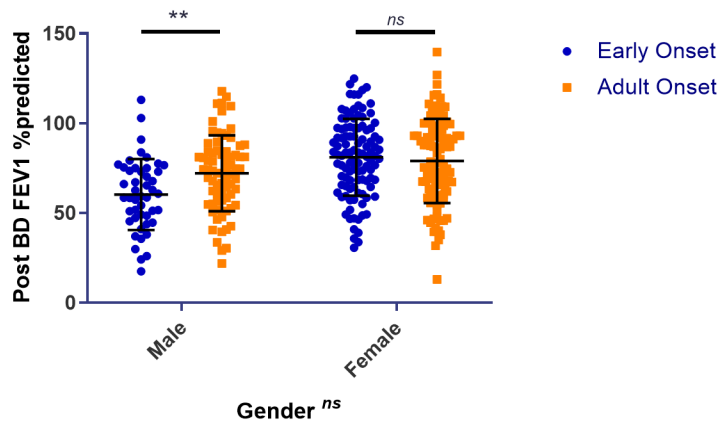
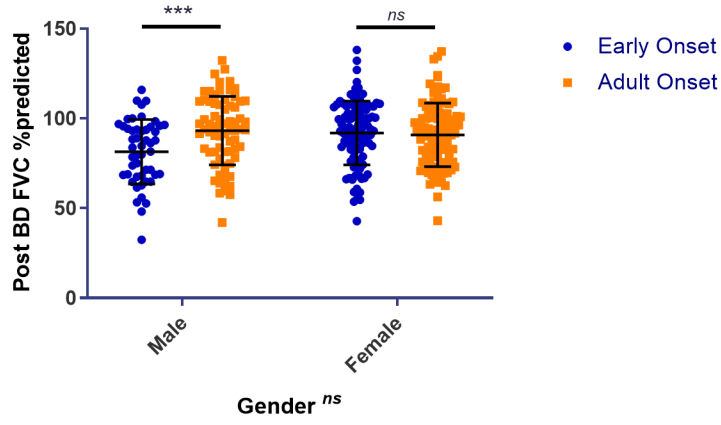
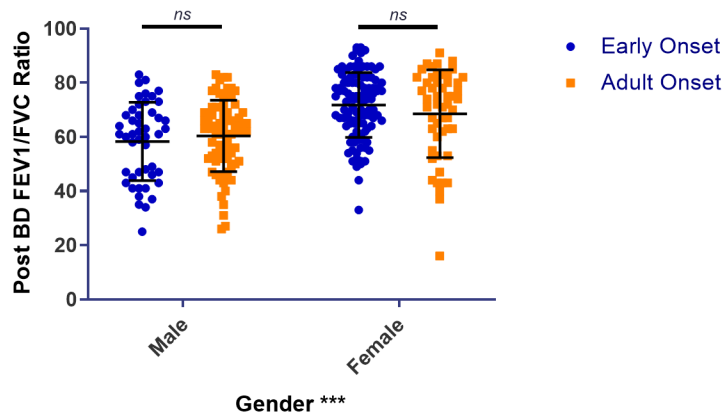
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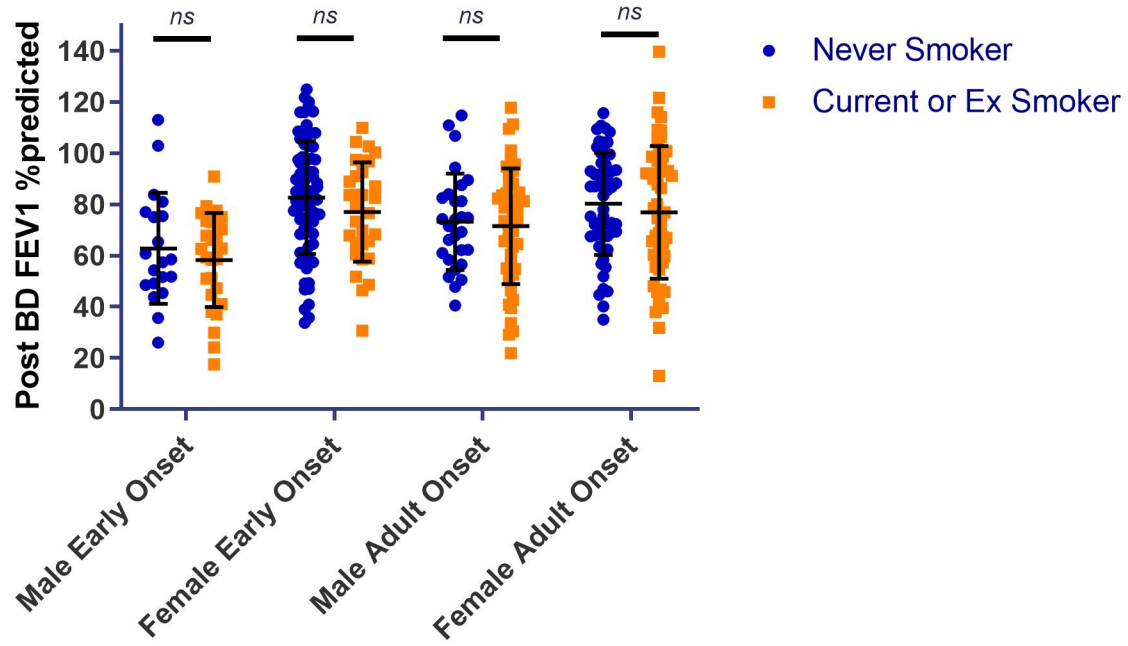
	<b>WATCH Study – Difficult Asthma (N= 501)</b>		<b>IOWBC – Mild Asthma (Overall cohort = 1456)</b>	<b>EOSA Cohort – Mild Asthma (N=69)</b>	
<b>Mean Age (years)</b>	50.6 (15.8)		18.0 (0.6)	38.3 (12.6)	
	<b>Early-Onset</b>	<b>Adult-onset</b>	<b>Early-Onset (Ever Asthmatic in 1st 18-years; N = 359)</b>	<b>Early-Onset</b>	<b>Adult-onset</b>
<b>%Male</b>	40.2%	59.8%	50.7%	74.1%	25.9%
<b>%Female</b>	53.2%	46.8%	49.3%	53.9%	46.2%
			<b>Early-Onset (Currently Asthmatic at 18-years; N =231)</b>		
<b>%Male</b>			44.6%		
<b>%Female</b>			55.4%		

90 *Table E8 Notes: Early-onset <18-years; Adult-onset ≥18-years. WATCH study = Wessex AsThma CoHort of difficult asthma,*91 *IOWBC = Isle of Wight Whole Population Birth Cohort, EOSA = Epigenetics of Severe Asthma study, mild cohort.*

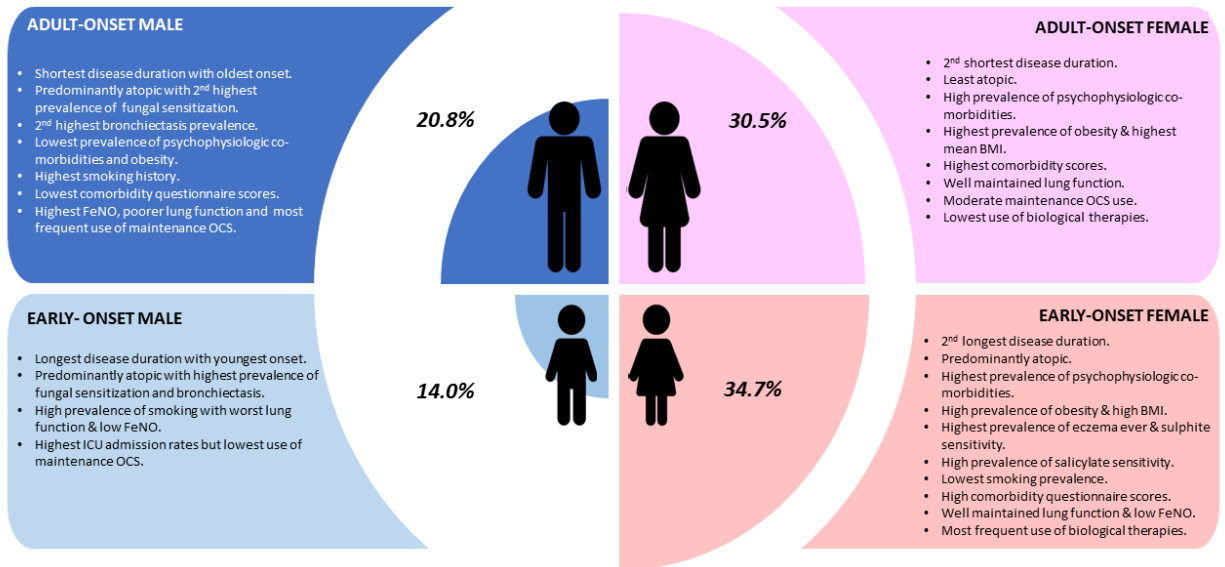
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