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Asthma prescribing according to Arg16Gly beta-2 genotype: a randomized trial in adolescents

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Key Words:	genotype, beta-2 gene polymorphisms, paediatric asthma, asthma clinical trials, Asthma Quality of Life
Abstract:	<p>Introduction: The A allele of rs1042713 (Arg16 amino acid) in the beta-2 (β2) adrenoreceptor is associated with poor response to long-acting β2-agonist (LABA) in young people with asthma. Our aim was to assess whether the prescribing of second line controller with LABA or a leukotriene receptor antagonist (LTRA) according to Arg16Gly genotype would result in improvements in pediatric asthma-related quality of life questionnaire (PAQLQ).</p> <p>Methods: We performed a pragmatic randomized controlled trial (RCT)</p>

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	<p>via a primary care clinical research network covering England and Scotland. We enrolled participants aged 12-18 years with asthma taking inhaled corticosteroids. A total of 241 participants (mean (SD) age 14.7 years (1.91)) were randomized (1:1) to receive personalized care (genotype directed prescribing) or standard guideline care. Following 4-week run-in participants were followed for 12-months. The primary outcome measure was change in PAQLQ. Asthma control, asthma exacerbation frequency and healthcare utilization were secondary outcomes.</p> <p>Results: Genotype directed prescribing resulted in an improvement in PAQLQ compared to standard care 0.16, (95%CI 0.00-0.31; p=0.049), although this improvement was below the pre-determined clinical threshold of 0.25. The AA genotype was associated with a larger improvement in PAQLQ with personalized versus standard care 0.42, (95%CI 0.02-0.81; p=0.041).</p> <p>Conclusion: This is the first RCT demonstrating that genotype driven asthma prescribing is associated with a significant improvement in a clinical outcome compared to standard care. Adolescents with the AA homozygous genotype benefited most. The potential role of such β2-adrenoceptor genotype directed therapy in younger and more severe childhood asthma warrants further exploration.</p>

Asthma prescribing according to Arg16Gly beta-2 genotype: a randomized trial in adolescents

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44
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46
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48
49 Statistical analysis: P.R.
50
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53
54 Supervision: B.L., SM.
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56
57 All authors contributed to interpretation of the data, participated in the writing of the
58
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60

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Take Home Message

Personalized prescribing in adolescents with asthma demonstrated that beta-2 adrenoreceptor genotype directed treatment results in a small but significant improvement in PAQLQ. Beta-2 adrenoreceptor genotype guided treatment requires further investigation.

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Abstract

Introduction The A allele of rs1042713 (Arg16 amino acid) in the beta-2 (β 2) adrenoreceptor is associated with poor response to long-acting β 2-agonist (LABA) in young people with asthma. Our aim was to assess whether the prescribing of second line controller with LABA or a leukotriene receptor antagonist (LTRA) according to Arg16Gly genotype would result in improvements in pediatric asthma-related quality of life questionnaire (PAQLQ).

Methods We performed a pragmatic randomized controlled trial (RCT) via a primary care clinical research network covering England and Scotland. We enrolled participants aged 12-18 years with asthma taking inhaled corticosteroids. A total of 241 participants (mean (SD) age 14.7 years (1.91)) were randomized (1:1) to receive personalized care (genotype directed prescribing) or standard guideline care. Following 4-week run-in participants were followed for 12-months. The primary outcome measure was change in PAQLQ. Asthma control, asthma exacerbation frequency and healthcare utilization were secondary outcomes.

Results Genotype directed prescribing resulted in an improvement in PAQLQ compared to standard care 0.16, (95%CI 0.00-0.31; p=0.049), although this improvement was below the pre-determined clinical threshold of 0.25. The AA genotype was associated with a larger improvement in PAQLQ with personalized versus standard care 0.42, (95%CI 0.02-0.81; p=0.041).

Conclusion This is the first RCT demonstrating that genotype driven asthma prescribing is associated with a significant improvement in a clinical outcome compared to standard care. Adolescents with the AA homozygous genotype benefited most. The potential role of such β 2-

25 adrenoceptor genotype directed therapy in younger and more severe childhood asthma

26 warrants further exploration.

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32 Beta-2 gene polymorphisms

33 Paediatric asthma

34 Asthma clinical trials

35 Asthma Quality of life

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38 **Abbreviations**

39	ACQ:	Asthma control questionnaire
40	ADRB2:	β2 adrenergic receptor
41	PAQLQ:	Pediatric asthma quality of life questionnaire
42	Arg:	Arginine
43	CI:	Confidence interval
44	CRN:	Clinical research network
45	<u>GINA</u>	<u>Global Initiative for Asthma</u>
46	Gly:	Glycine
47	HTA:	Health technology assessment
48	ICS:	Inhaled corticosteroids
49	IMD:	Index of Multiple Deprivation
50	LABA:	Long-acting β2 agonists
51	LTRA:	Leukotriene receptor antagonist
52	MCID:	Minimum clinically important difference
53	NiHR:	National institute for health research
54	PiCA:	Pharmacogenomics in childhood asthma
55	RCT:	Randomized controlled trial
56	SNP:	Single nucleotide polymorphism
57	TRuST:	Tayside randomisation system
58	UKCRN:	United Kingdom Clinical Research Network

60 Introduction

61 Asthma is the most common chronic condition affecting children [1]. It is associated with
62 substantial health and quality of life burden for the patient as well as significant healthcare
63 expenditure globally [2]. Childhood asthma is treated in a step-wise approach using controller
64 medications, initially with inhaled corticosteroids (ICS) and if symptoms are not subsequently
65 well controlled then addition of either inhaled long-acting β 2 agonist (LABA) or a leukotriene
66 receptor antagonist (LTRA), or by a further increase in ICS dose [3].

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68 There is a wide degree of heterogeneity in response to treatment amongst young people with
69 asthma, with estimates that 60-80% of the observed variance between individuals could be
70 due to genetic differences [4]. There has been particular interest in a variation in the gene
71 encoding for the β 2 adrenergic receptor (*ADRB2*) at position 16 (rs1042713) resulting in an
72 allelic substitution from glycine to arginine (Gly16Arg). The homozygous AA variant is found in
73 approximately 15% of people and has been associated with poor response to ICS-LABA
74 controller therapy in young people [5-7]. These findings have not been widely replicated in
75 adult studies aside from the demonstration of greater bronchoprotective subsensitivity with
76 the A allele in response to long acting β -agonist therapy [8-11]. It is hypothesized that, for
77 young people with the A genotype, regular ICS-LABA use results in agonist induced down
78 regulation and associated uncoupling of the β 2 receptor thus impairing the efficacy of the
79 medication [12, 13].

80
81 A meta-analysis from the Pharmacogenomics in Childhood Asthma (PiCA) consortium
82 comprising 4226 children showed a 34% elevated risk of asthma exacerbation for each copy of
83 the A allele in young people with ICS-LABA controller treatment, with at least one copy of A

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2 84 allele being present in 62.8% of people [7]. One prospective study showed that use of a LTRA
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5 85 instead of LABA in young people homozygous for AA reduced school absence and improved
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7 86 asthma symptom and quality of life scores [14]. It is thus important to test whether Arg16Gly
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10 87 genotype directed therapy (personalized medicine) in adolescents with asthma leads to
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12 88 improvement in quality of life.
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18 90 The principal aim was to test our hypothesis that prescribing of second line asthma controller
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20 91 medication (LABA or LTRA) according to Arg16Gly genotype compared to standard care
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23 92 provided according to the British Thoracic Society (BTS) guidelines would result in an
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25 93 improvement in quality of life determined by standardized pediatric asthma quality of life
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27 94 questionnaire (PAQLQ) in 12-18 year olds with asthma. Secondary aims included assessing the
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29 95 effect of genotype directed prescribing on: (i) asthma control (validated asthma control
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31 96 questionnaire (ACQ-6)); (ii) exacerbation frequency (requirement for oral steroids) and; (iii)
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33 97 health care utilization (non-routine primary-care review, emergency department attendance or
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35 98 hospital admission).
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43 100 Some results have been previously reported in the form of an abstract [15].
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50 103 **Methods**

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52 104 ***Subjects***

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55 105 Our trial consisted of participants of either sex aged 12-18 years with: (i) a documented
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57 106 physician diagnosis of asthma; (ii) taking inhaled corticosteroid (ICS) with or without the
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60 107 additional second-line controllers (LABA or LTRA). The target population was adolescents

whose asthma was managed in primary care in England and Scotland. Exclusions were: (i) known contraindication to LABA or LTRA; (ii) On step 4 of BTS guidelines (e.g. use of theophylline based controller medication such as Uniphyllin); (iii) presence of other major airway or lung disease (other than asthma); (iv) pregnancy or lactation; (v) participation in another clinical trial; (vi) inability to provide saliva / buccal cells for genotyping. ~~We excluded participants with a known contraindication to LTRA or LABA as well as participants with clinically significant co-morbidities. A full list of inclusion and exclusion criteria is provided in the study protocol (supplementary material).~~

Study design

For this two-arm pragmatic randomized controlled trial (RCT), participants were recruited from throughout England and Scotland. The study duration was 13-months, consisting of a 4-week run-in period and 12-months of follow-up.

Participants were principally recruited through primary care via the clinical research network (CRN) across England and Scotland as well as the patient databases BREATHE and PAGES. Informed consent and assent were obtained online or by telephone with follow-up written consent. The study followed the Children's Research Network standard operating procedures, Health Research Agency guidelines and the Nuffield Council on Bioethics: Ethical issues obtaining informed consent (2015). Participant's aged 12-18 consented independently whilst parental consent was sought in addition for those 15 years and younger. The trial was sponsored by the University of Sussex (approval December 2014) and ethical approval was obtained from the East of Scotland Research Ethics Committee (15/ES/0007, approval March 2015). The trial was registered on the UK Clinical Research Network (UKCRN) website with

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2 132 details made available to the public before the recruitment of the first participant. This trial is
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5 133 registered with ClinicalTrials.gov NCT02758873.
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10 135 Participants were randomized 1:1 ratio to personalized care (rs1042713 SNP based prescribing)
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12 136 or standard care by a web-based system, TRuST (Tayside Randomisation SysTem). Participants
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15 137 were allocated as per block randomization with no stratification or minimization. The
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17 138 personalized care group were prescribed asthma controller medication on the basis of their
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19 139 Arg16Gly genotype, AA or AG receiving montelukast (LTRA) and GG receiving salmeterol
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22 140 (LABA). The standard care group were prescribed controller medication based on the current
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24 141 ~~BT~~~~ritish~~~~Thoracic~~~~Society~~ guidelines. Neither group nor the study team was blinded to group
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27 142 allocation or prescribed medication.
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32 144 Participants undertook a 4-week run-in period where they were asked to use only ICS as their
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34 145 controller medication at the previously prescribed dose. Reliever medication was used by each
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36 146 participant as required. Outcomes were measured at baseline, following completion of run-in
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39 147 and 3, 6, 9 and 12-months. Study questionnaires were completed online or over the phone.
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42 148 Medications were prescribed by the participant's GP.
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46 150 Change to the asthma control questionnaire (ACQ-6) score was used to determine the
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48 151 participant's controller treatment with a score ≥ 1.0 [16] or need for oral corticosteroid
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51 152 triggering escalation. The personalized care group were prescribed asthma controller
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54 153 medication on the basis of their Arg16Gly genotype, AA or AG genotypes receiving LTRA and
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56 154 GG receiving LABA. The standard care group were prescribed controller medication based on
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58 155 the ~~BT~~~~ritish~~~~Thoracic~~~~Society~~ guidelines. A stable or decrease in ACQ score resulted in
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156 continuation of current treatment.

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158 DNA collection kits were posted to participants with instructions and a paid return envelope.
159 Saliva samples were collected using a commercially available pot (GeneFiX™ DNA saliva
160 collector, Isohelix.com). DNA was prepared with the Isohelix GeneFiX saliva prep DNA kit. DNA
161 extraction and Arg16Gly genotype status was determined at the University of Dundee, Division
162 of Population and Health Genomics, using TaqMan-based allelic discrimination assays on an
163 ABI 7900 Sequence Detection System (Applied Biosystems, Foster City, USA) as previously
164 described [17].

165
166 Outcomes
167 The primary outcome was the change in pediatric asthma quality of life questionnaire (PAQLQ)
168 [18] from completion of the run-in to completion of the study at 12-months. Secondary
169 outcomes were change in asthma control questionnaire (ACQ-6) score, health care utilization
170 for asthma management (non-routine primary care review, emergency department
171 attendance or hospital admission) as well as exacerbation frequency (courses of oral
172 corticosteroid). Adverse events were recorded as per Health Research Authority guidelines.

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174 Analysis
175 A change of 0.5 units on the PAQLQ is considered to represent the minimal clinically important
176 difference (MCID) [19]. We expected to see a 0.25 improvement in PAQLQ at 12 months in the
177 standard care group with the improvements estimated on the basis of genotype frequency
178 with a projected improvement of: 0.5 in the GG (40%); 0.25 in the AG (35%) and 0 in the AA
179 genotype (15%). The calculated sample size, in order to detect a clinically relevant threshold of
180 0.25 units ($0.5 - 0.25 = 0.25$) in the primary outcome of PAQLQ score ($SD = 1.0$; $\alpha = 0.05$; 90%

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2 181 power) was 100 participants in each group. To allow for a 15% attrition rate, the recruitment
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5 182 target was increased to 120 participants in each group.
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10 184 Analyses comparing personalized and standard care were completed as pre-specified in the
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12 185 statistical analysis plan. All analyses were performed on intention to treat population i.e. by
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14 186 group randomized. Data for continuous outcome measures were assessed for normality prior
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17 187 to analysis. Transformations of the outcome variables were used where necessary if they were
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19 188 not normally distributed. If data were normally distributed, outcome measures were assessed
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22 189 using mixed model repeated measure analysis, adjusted for the corresponding baseline values
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24 190 and group allocation as fixed effects. Models used all available data from the end of the run-in
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27 191 period. Where data was not normally distributed and could not be transformed into a normal
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29 192 distribution, data was analyzed using non-parametric methods. Subgroup analyses were
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32 193 performed on participants with the Arg16Gly status AA. A two-sided P value of <0.05 was taken
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34 194 to be significant for all analyses. SAS software (version 9.4, SAS Institute Inc. Cary, NC, USA)
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37 195 was used for all statistical analyses. PACT conforms to CONSORT 2010 guidelines on RCT
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39 196 reporting.
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46 199 **Results**

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49 200 Between February 9, 2016 and April 25, 2018, 247 participants were consented and 241
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51 201 randomized before entering the 4-week run-in period (figure 1). Participants were randomized
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54 202 to either personalized care (n=121) or standard care (n=120) for their asthma controller
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56 203 therapy. Baseline demographics and clinical characteristics were broadly similar between those
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58
59 204 receiving personalized or standard care with a mean age of 14.7 years across the two groups
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205 (table 1). There are however important differences between the groups with ~~was~~ a greater

proportion of adolescents receiving ICS, LABA and LTRA combination therapy at baseline in the standard care group (15% vs 6.6%) indicating a possible increased asthma severity in this group, and a lower prevalence of the AA genotype in the personalized care group (9.9% vs 15.0%), possibly impairing the overall clinical benefit of personalized medication prescribing in this group.-

Completion of the run-in period (ICS only) resulted in 0.1 mean improvement in PAQLQ compared to baseline in both groups. A mean improvement in PAQLQ total score compared to end of run-in was observed in both the personalized and standard care groups (table 2).

Prescription of asthma controller medication as per Arg16Gly genotype SNP status (personalized care) resulted in an improvement in mean PAQLQ compared to standard care (0.16, 95% CI 0.00-0.31; $p=0.049$; table 2; figure 2a; figure 3) however, the difference was below the pre-determined clinical threshold of 0.25. The PAQLQ domains of emotional function and activity limitation score had the greatest mean difference in change with personalized care.

Sub-group analysis of the children with the homozygous AA genotype ($n=27$) demonstrated an improvement in mean PAQLQ that exceeded the clinical threshold of 0.25 in those receiving personalized compared to standard care (0.42, 95% CI 0.02-0.813; $p=0.04$; table 2; figure 2b).

There were no adverse or serious adverse events reported throughout the duration of the trial.

A total of 28 (11.6%) participants experienced an asthma exacerbation (requirement for oral steroids) during 12-month follow-up, with numerically lower rates reported in the personalized care (8.3%) compared to standard care group (15.3%) ($p=0.08$). There was also a trend toward

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2 231 increased time to exacerbation in the personalized care (225.7 days) compared to the standard
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5 232 care group (141.5 days) (p=0.102). There was a similar improvement in ACQ score from the end
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7 233 of run-in when mean change was compared in the personalized care (0.42) and standard care
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9 234 groups (0.47) (p=0.18). There was no association between personalized asthma controller
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11 235 directed prescribing and health care utilization or the number of asthma medications
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13 236 prescribed with a small increase in the mean number prescribed in the personalized care (0.3)
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15 237 and standard care groups (0.2) (p=0.36) (table 3). There was no association between
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17 238 personalized asthma controller directed prescribing and ACQ score, health care utilization or
18
19 239 number of asthma medicines prescribed (table 3).
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242 **Discussion**
243 The PACT study is the first prospective RCT assessing the efficacy of genotype-directed
244 prescribing in adolescent asthma. Prescription of second line controller medication with LABA
245 or LTRA according to Arg16Gly genotype resulted in a statistically significant improvement in
246 primary clinical outcome of PAQLQ, although the magnitude of improvement amounted to
247 0.16 and was below the a priori clinical threshold of 0.25. The quality of life benefit seen in
248 adolescents with the homozygous AA genotype was 0.42, exceeding the clinically significant
249 PAQLQ threshold.

250
251 Our findings are consistent with the only previous trial conducted examining the effects of
252 LABA in relation to Arg16Gly genotype in children. That proof-of-concept study focused on
253 previously genotyped asthmatic children who were all homozygous for the AA variant. A total
254 of 62 children were randomized to receive either ICS and LABA or ICS and LTRA and were
255 followed-up for 12-months. Children treated with a LTRA compared to LABA had a clinically

relevant mean improvement in PAQLQ scores amounting to 0.53 as well as reduced school absences and use of rescue medication in comparison to those treated with LABA. [14] In-keeping with these findings, adolescents with the AA homozygous genotype benefitted most from genotype-directed prescribing in our current study.

Both RCTs are underpinned by a large meta-analysis of observational studies comprising five childhood asthma cohorts demonstrating an increased risk of exacerbations with each copy of the A allele amounting to a 34% (95% CI 15-50) difference for asthmatic children treated with ICS-LABA therapy [7]. In this study, we report a non-significant numerical trend toward decreased exacerbation frequency in the genotype directed treatment group (8.3% vs. 15.3%, $p=0.08$). The low exacerbation frequency (11.6%) within our well-controlled population recruited for this study is a likely confounding factor as to why this well described association did not reach significance. A 2018 systematic review further assessed the role of Arg16Gly genotype variation on LABA response in asthma, confirming that the contribution of genetics to LABA response is more consistently shown in children compared to adults [20]. It is hypothesized that this could relate to the altered phenotype of children's asthma with a greater emphasis of atopy in its pathogenesis as well as reduced duration of chronic airway inflammation and airway wall rigidity [21]. It is thus possible that a greater mean improvement in PAQLQ could be achieved with this genotype-directed intervention in younger children, especially in those with more severe disease.

A conspicuous observation from the study was that both the personalized and standard care groups had improved PAQLQ and ACQ at final follow-up, this notable 'trial effect' is well described [22]. A component of this known as the 'care effect' could result from participants in both arms of the PACT trial having more regular face-to-face contact with primary care

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2 281 healthcare practitioners. The frequent telephone or e-mail communication with the study
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5 282 team as per the study protocol may have altered health behavior - the Hawthorne effect [23].
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7 283 It is also conceivable that by virtue of regularly completing the PAQLQ and ACQ questionnaires
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10 284 adolescents may have developed improved asthma symptom awareness, promoting self-
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12 285 education about asthma, triggers and the benefits of controller treatment and improved
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14 286 adherence. This, potential 'trial effect', may explain how the mean improvement in PAQLQ
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17 287 exceeded the MCID in both groups which may in-turn have contributed to a smaller than
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20 288 anticipated response to genotype-directed therapy.
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27 291 Our study has some limitations. The lower than expected effect of personalized medicine on
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30 292 PAQLQ score could partly be explained by the excellent symptom control at baseline with very
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32 293 good PAQLQ and ACQ scores demonstrated, leaving limited room for further improvement.
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34 294 This explanation is supported by the large number of adolescents who did not experience an
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37 295 exacerbation (88.4%) and did not require any non-routine healthcare utilization for their
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39
40 296 asthma (71.4%) during 12-month follow-up. The good baseline control on ICS treatment
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42 297 following the run-in period resulted in 65% of adolescents in the personalized medicine group
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44 298 not actually experiencing specific genotype based prescribing e.g. add on of LABA or LTRA thus
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46
47 299 diminishing the possibility of the benefit of personalized begin proven. Future studies aiming to
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49 300 identify the potential role of Arg16Gly genotype directed therapy may require selective
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52 301 recruitment of children with poorly controlled asthma. Differences between the groups could
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54 302 also explain the lower than anticipated response to personalized treatment. The greater
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56 303 proportion of adolescents receiving ICS, LABA and LTRA combination therapy at enrolment in
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58
59 304 the standard care group (15% vs 6.6%) could indicate potentially increased disease severity at
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305 baseline. A further contributing factor could be the lower than anticipated number of

adolescents with the significant AA genotype in the personalized medicine group (9.9%) compared to the standard care group (15%) and a previous large population meta-analysis (16.1%) [7].

Ideally, the criteria for asthma diagnosis should be defined in accordance with the Global Initiative for Asthma (GINA) guidelines and documented for each patient involved in the study. By recruiting patients with a physician diagnosis of asthma from primary care, where there may not be routine access to spirometry or the capability to perform bronchodilator reversibility, the accuracy of asthma diagnosis is impaired which is a notable limitation. However, as the vast majority of adolescents with asthma are managed in this setting this approach assesses the pragmatic real world efficacy of personalised asthma prescribing. The lack of ethnic diversity within the study participants (88.8% Caucasian) is a potential limitation. There is however, reason to believe our results could be generalizable to other populations given the finding of increased rate of exacerbation with LABA use with presence of the A allele amongst cohorts of young people of differing ethnicities [7].

An important element of the PACT study is in relation to the novel study methodology employed, utilizing telephone and online contact for consent and data collection along with study directed prescribing through the patients' GP without direct face-to-face contact with study team members at any point. This is unique in the investigation of young people's asthma. Replication of this methodology may help investigators to conduct trials in a safe manner during the Covid-19 pandemic. The significant associated cost reduction has important implications for future research studies, with the cost per patient of \$1495 for the PACT study substantially lower compared to a mean cost of \$4630 per patient for RCT's funded by the

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2 330 NIHR (National Institute for Health Research) Health Technology Assessment (HTA) program
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5 331 between 2000-2005 [24].
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10 333 Further areas need to be explored more fully to understand the potential value of Arg16Gly
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12 334 genotype directed prescribing in the treatment of children’s asthma especially those with more
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15 335 severe disease. On the basis of our findings it is important to re-evaluate our study question in
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17 336 a less well-controlled sample including younger children with suggestions that the benefits of
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20 337 Arg16Gly genotype based prescribing are more pronounced in this group. It is hoped that we
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22 338 will have a greater understanding following publication of the PUFFIN trial, an in progress
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25 339 multi-center Dutch study exploring Arg16Gly genotype based prescribing in 6-17 year-olds with
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27 340 asthma utilizing asthma control test as the primary outcome measure [25].
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32 342 **Conclusion**
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35 343 In this 12-month trial, asthma controller prescribing on the basis of Arg16Gly beta-2 receptor
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37 344 genotype resulted in a small but significant improvement in PAQLQ in adolescents compared
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40 345 to standard care, however this was below the expected clinical threshold. A clear benefit was
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42 346 demonstrated for those with the AA homozygous genotype (15% of the population). We
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45 347 recommend further prospective randomized studies to help identify the potential clinical utility
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47 348 of personalized prescribing in young people’s asthma.
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51 350
52 351 **Role of the Funder/Sponsor:** The funders had no role in the design and conduct of the study;
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54 352 collection, management, analysis an interpretation of the data; preparation, review, or
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56
57 353 approval of the manuscript; and decision to submit the manuscript for publication.
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Figure legends

Figure 1 - Trial profile

Figure 2a - Pediatric asthma quality of life questionnaire total score

Data represented includes the median, 25th centile, 75th centile, lowest value and highest value. PAQLQ – Pediatric Asthma Quality of Life Questionnaire.

Figure 2b - Pediatric asthma quality of life questionnaire total score for A/A genotype

Data represented includes the median, 25th centile, 75th centile, lowest value and highest value. PAQLQ – Pediatric Asthma Quality of Life Questionnaire.

Figure 3 - Pediatric Asthma quality of life questionnaire total score change from baseline to 12 months follow-up

MCID – minimum clinically important difference

Table 1 – Baseline demographics and clinical characteristics

	Personalized care N=121	Standard care N=120
Age, years	14.6 (1.95)	14.7 (1.87)
Sex		
Male	57 (47.1%)	52 (43.3%)
Female	64 (52.9%)	68 (56.7%)
Genotype		
GLY/GLY (G/G)	53 (43.8%)	48 (40%)
ARG/GLY (A/G)	54 (44.6%)	53 (44.2%)
ARG/ARG (A/A)	12 (9.9%)	18 (15%)
Missing*	2 (1.7%)	1 (0.8%)
Ethnicity		
White	111 (91.7%)	103 (85.8%)
Black	0 (0%)	3 (2.5%)
Asian	1 (0.8%)	3 (2.5%)
Other	9 (7.4%)	11 (9.2%)
Baseline medications		
ICS	78 (64.5%)	79 (65.8%)
ICS + LABA	30 (24.8%)	19 (15.8%)
ICS + LTRA	5 (4.1%)	4 (3.3%)
ICS + LABA + LTRA	8 (6.6%)	18 (15%)
Healthcare use in previous month	14 (11.6%)	16 (13.3%)

* Participants withdrew from the study without providing saliva specimen

Data are mean (SD) or n (%)

ICS - Inhaled corticosteroid, LABA - Long-acting β 2-agonist, LTRA - leukotriene receptor antagonist

Table 2 – Pediatric asthma quality of life questionnaire (PAQLQ) score change

	End of run-in values	12 month values	Mean change	Overall mean difference in change *	
	Personalized Standard	Personalized Standard	Personalized Standard	95% CI	P value
PAQLQ total score	5.97 (5.80-6.14)	6.44 (6.31-6.57)	0.45 (0.27-0.63)	0.16 (0.00-0.31)	0.049
PAQLQ emotional function score	5.84 (5.65-6.02)	6.32 (6.19-6.45)	0.46 (0.28-0.65)	0.23 (0.04-0.42)	0.02
PAQLQ environment stimuli score	5.91 (5.71-6.10)	6.44 (6.32-6.56)	0.53 (0.35-0.72)	0.12 (-0.08-0.32)	0.25
PAQLQ activity limitation score	5.69 (5.47-5.90)	6.22 (6.06-6.39)	0.50 (0.26-0.74)	0.14 (0.00-0.28)	0.04
PAQLQ symptom score	5.88 (5.65-6.10)	6.42 (6.25-6.59)	0.49 (0.26-0.73)	0.16 (-0.02-0.33)	0.08
PAQLQ total score (A/A)	5.82 (5.60-6.04)	6.35 (6.18-6.52)	0.48 (0.24-0.71)	0.42 (0.02-0.81)	0.04 **
	5.77 (5.12-6.42)	6.36 (6.14-6.59)	0.64 (0.06-1.22)		

Data are mean and 95% CI unless otherwise indicated.

PAQLQ – standardized pediatric asthma quality of life questionnaire.

* adjusted for treatment, genotype and baseline values.

** adjusted for treatment, genotype, baseline values and SAP characteristics (sex, age, site and Index for Multiple Deprivation (IMD))

Table 3 – Secondary outcomes measures

	Personalized care	Standard care	Overall mean difference in change*	
			Hazard Ratio (95% CI)	p-value
Any exacerbation, number (%)	10 (8.3%)	18 (15.0%)	0.52 (0.24-1.36)	0.10
Time to first exacerbation, days	225.7 (121.3-330.1)	141.5 (78.7-204.3)	2.11 (0.91-4.87)	0.08
No health care utilization, number (%)	87 (71.9%)	85 (70.8%)	0.98 (0.56-1.72)	0.73
Number of medications, mean change	0.3 (0.15-0.42)	0.2 (0.07-0.29)	1.71 (0.85-3.45)	0.36
PAQLQ-6, mean change	-0.42 (-0.60-0.42)	-0.47 (-0.66-0.27)	-0.10 (-0.25-0.05)	0.18

Data are mean (SD) or n (%).

PAQLQ – standardized pediatric asthma quality of life questionnaire.

Asthma prescribing according to Arg16Gly beta-2 genotype: a randomized trial in adolescents

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Study concept and design: C.J.P., S.T., F.H., H.S., B.L., S.M.

Patient recruitment, acquisition of data and database management: K.P., F.H.

Genotyping: R.T.

Statistical analysis: P.R.

Drafting of manuscript and critical revision: T.R., C.J.P., S.T., B.L., S.M.

Supervision: B.L., SM.

All authors contributed to interpretation of the data, participated in the writing of the manuscript and have approved the final version for submission.

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Disclosures:

B.L. reports other from GSK, grants, personal fees and non-financial support from AstraZeneca, grants, personal fees and non-financial support from Chiesi, personal fees from Novartis, grants, personal fees and non-financial support from Boehringer Ingelheim, personal fees from Dr Reddys, personal fees from Sandoz, personal fees from Cipla, personal fees from Glenmark, grants, personal fees and non-financial support from Teva, during the conduct of the study; personal fees from Lupin, grants and personal fees from Sanofi Regeneron, personal fees from Vectura, outside the submitted work; and Son of BJL is employee of AstraZeneca. J.G. reports personal fees from AstraZeneca, personal fees from GSK, personal fees from Medimmune, personal fees from BV Pharma, during the conduct of the study.

Take Home Message

Personalized prescribing in adolescents with asthma demonstrated that beta-2 adrenoreceptor genotype directed treatment results in a small but significant improvement in PAQLQ. Beta-2 adrenoreceptor genotype guided treatment requires further investigation.

Abstract

Introduction The A allele of rs1042713 (Arg16 amino acid) in the beta-2 (β 2) adrenoreceptor is associated with poor response to long-acting β 2-agonist (LABA) in young people with asthma.

Our aim was to assess whether the prescribing of second line controller with LABA or a leukotriene receptor antagonist (LTRA) according to Arg16Gly genotype would result in improvements in pediatric asthma-related quality of life questionnaire (PAQLQ).

Methods We performed a pragmatic randomized controlled trial (RCT) via a primary care clinical research network covering England and Scotland. We enrolled participants aged 12-18 years with asthma taking inhaled corticosteroids. A total of 241 participants (mean (SD) age 14.7 years (1.91)) were randomized (1:1) to receive personalized care (genotype directed prescribing) or standard guideline care. Following 4-week run-in participants were followed for 12-months. The primary outcome measure was change in PAQLQ. Asthma control, asthma exacerbation frequency and healthcare utilization were secondary outcomes.

Results Genotype directed prescribing resulted in an improvement in PAQLQ compared to standard care 0.16, (95%CI 0.00-0.31; $p=0.049$), although this improvement was below the pre-determined clinical threshold of 0.25. The AA genotype was associated with a larger improvement in PAQLQ with personalized versus standard care 0.42, (95%CI 0.02-0.81; $p=0.041$).

Conclusion This is the first RCT demonstrating that genotype driven asthma prescribing is associated with a significant improvement in a clinical outcome compared to standard care. Adolescents with the AA homozygous genotype benefited most. The potential role of such β 2-

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3 25 adrenoceptor genotype directed therapy in younger and more severe childhood asthma
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5 26 warrants further exploration.
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10 28 **Abstract word count: 249**
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12 29
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15 30 **Key words:**
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17 31 Genotype
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22 33 Paediatric asthma
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Abbreviations

39	ACQ:	Asthma control questionnaire
40	ADRB2:	β 2 adrenergic receptor
41	PAQLQ:	Pediatric asthma quality of life questionnaire
42	Arg:	Arginine
43	CI:	Confidence interval
44	CRN:	Clinical research network
45	GINA	Global Initiative for Asthma
46	Gly:	Glycine
47	HTA:	Health technology assessment
48	ICS:	Inhaled corticosteroids
49	IMD:	Index of Multiple Deprivation
50	LABA:	Long-acting β 2 agonists
51	LTRA:	Leukotriene receptor antagonist
52	MCID:	Minimum clinically important difference
53	NiHR:	National institute for health research
54	PiCA:	Pharmacogenomics in childhood asthma
55	RCT:	Randomized controlled trial
56	SNP:	Single nucleotide polymorphism
57	TRuST:	Tayside randomisation system
58	UKCRN:	United Kingdom Clinical Research Network

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Introduction

Asthma is the most common chronic condition affecting children [1]. It is associated with substantial health and quality of life burden for the patient as well as significant healthcare expenditure globally [2]. Childhood asthma is treated in a step-wise approach using controller medications, initially with inhaled corticosteroids (ICS) and if symptoms are not subsequently well controlled then addition of either inhaled long-acting β 2 agonist (LABA) or a leukotriene receptor antagonist (LTRA), or by a further increase in ICS dose [3].

There is a wide degree of heterogeneity in response to treatment amongst young people with asthma, with estimates that 60-80% of the observed variance between individuals could be due to genetic differences [4]. There has been particular interest in a variation in the gene encoding for the β 2 adrenergic receptor (*ADRB2*) at position 16 (rs1042713) resulting in an allelic substitution from glycine to arginine (Gly16Arg). The homozygous AA variant is found in approximately 15% of people and has been associated with poor response to ICS-LABA controller therapy in young people [5-7]. These findings have not been widely replicated in adult studies aside from the demonstration of greater bronchoprotective subsensitivity with the A allele in response to long acting β -agonist therapy [8-11]. It is hypothesized that, for young people with the A genotype, regular ICS-LABA use results in agonist induced down regulation and associated uncoupling of the β 2 receptor thus impairing the efficacy of the medication [12, 13].

A meta-analysis from the Pharmacogenomics in Childhood Asthma (PiCA) consortium comprising 4226 children showed a 34% elevated risk of asthma exacerbation for each copy of the A allele in young people with ICS-LABA controller treatment, with at least one copy of A

allele being present in 62.8% of people [7]. One prospective study showed that use of a LTRA instead of LABA in young people homozygous for AA reduced school absence and improved asthma symptom and quality of life scores [14]. It is thus important to test whether Arg16Gly genotype directed therapy (personalized medicine) in adolescents with asthma leads to improvement in quality of life.

The principal aim was to test our hypothesis that prescribing of second line asthma controller medication (LABA or LTRA) according to Arg16Gly genotype compared to standard care provided according to the British Thoracic Society (BTS) guidelines would result in an improvement in quality of life determined by standardized pediatric asthma quality of life questionnaire (PAQLQ) in 12-18 year olds with asthma. Secondary aims included assessing the effect of genotype directed prescribing on: (i) asthma control (validated asthma control questionnaire (ACQ-6)); (ii) exacerbation frequency (requirement for oral steroids) and; (iii) health care utilization (non-routine primary-care review, emergency department attendance or hospital admission).

Some results have been previously reported in the form of an abstract [15].

Methods

Subjects

Our trial consisted of participants of either sex aged 12-18 years with: (i) a documented physician diagnosis of asthma; (ii) taking inhaled corticosteroid (ICS) with or without the additional second-line controllers (LABA or LTRA). The target population was adolescents

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2 108 whose asthma was managed in primary care in England and Scotland. Exclusions were: (i)
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4 109 known contraindication to LABA or LTRA; (ii) On step 4 of BTS guidelines (e.g. use of
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6 110 theophylline based controller medication such as Uniphyllin); (iii) presence of other major
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8 111 airway or lung disease (other than asthma); (iv) pregnancy or lactation; (v) participation in
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10 112 another clinical trial; (vi) inability to provide saliva / buccal cells for genotyping.
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17 114 **Study design**

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19 115 For this two-arm pragmatic randomized controlled trial (RCT), participants were recruited from
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21 116 throughout England and Scotland. The study duration was 13-months, consisting of a 4-week
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23 117 run-in period and 12-months of follow-up.
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29 119 Participants were principally recruited through primary care via the clinical research network
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31 120 (CRN) across England and Scotland as well as the patient databases BREATHE and PAGES.
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33 121 Informed consent and assent were obtained online or by telephone with follow-up written
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35 122 consent. The study followed the Children’s Research Network standard operating procedures,
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37 123 Health Research Agency guidelines and the Nuffield Council on Bioethics: Ethical issues
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39 124 obtaining informed consent (2015). Participant’s aged 12-18 consented independently whilst
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41 125 parental consent was sought in addition for those 15 years and younger. The trial was
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43 126 sponsored by the University of Sussex (approval December 2014) and ethical approval was
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45 127 obtained from the East of Scotland Research Ethics Committee (15/ES/0007, approval March
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47 128 2015). The trial was registered on the UK Clinical Research Network (UKCRN) website with
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49 129 details made available to the public before the recruitment of the first participant. This trial is
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51 130 registered with ClinicalTrials.gov NCT02758873.
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Participants were randomized 1:1 ratio to personalized care (rs1042713 SNP based prescribing) or standard care by a web-based system, TRuST (Tayside Randomisation SysTem). Participants were allocated as per block randomization with no stratification or minimization. The personalized care group were prescribed asthma controller medication on the basis of their Arg16Gly genotype, AA or AG receiving montelukast (LTRA) and GG receiving salmeterol (LABA). The standard care group were prescribed controller medication based on the current BTS guidelines. Neither group nor the study team was blinded to group allocation or prescribed medication.

Participants undertook a 4-week run-in period where they were asked to use only ICS as their controller medication at the previously prescribed dose. Reliever medication was used by each participant as required. Outcomes were measured at baseline, following completion of run-in and 3, 6, 9 and 12-months. Study questionnaires were completed online or over the phone. Medications were prescribed by the participant's GP.

Change to the asthma control questionnaire (ACQ-6) score was used to determine the participant's controller treatment with a score ≥ 1.0 [16] or need for oral corticosteroid triggering escalation. The personalized care group were prescribed asthma controller medication on the basis of their Arg16Gly genotype, AA or AG genotypes receiving LTRA and GG receiving LABA. The standard care group were prescribed controller medication based on the BTS guidelines. A stable or decrease in ACQ score resulted in continuation of current treatment.

DNA collection kits were posted to participants with instructions and a paid return envelope. Saliva samples were collected using a commercially available pot (GeneFiX™ DNA saliva

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2 157 collector, Isohelix.com). DNA was prepared with the Isohelix GeneFiX saliva prep DNA kit. DNA
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5 158 extraction and Arg16Gly genotype status was determined at the University of Dundee, Division
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7 159 of Population and Health Genomics, using TaqMan-based allelic discrimination assays on an
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10 160 ABI 7900 Sequence Detection System (Applied Biosystems, Foster City, USA) as previously
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12 161 described [17].
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16 163 **Outcomes**

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19 164 The primary outcome was the change in pediatric asthma quality of life questionnaire (PAQLQ)
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22 165 [18] from completion of the run-in to completion of the study at 12-months. Secondary
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24 166 outcomes were change in asthma control questionnaire (ACQ-6) score, health care utilization
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27 167 for asthma management (non-routine primary care review, emergency department
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29 168 attendance or hospital admission) as well as exacerbation frequency (courses of oral
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32 169 corticosteroid). Adverse events were recorded as per Health Research Authority guidelines.
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36 171 **Analysis**

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39 172 A change of 0.5 units on the PAQLQ is considered to represent the minimal clinically important
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41 173 difference (MCID) [19]. We expected to see a 0.25 improvement in PAQLQ at 12 months in the
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44 174 standard care group with the improvements estimated on the basis of genotype frequency
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46 175 with a projected improvement of: 0.5 in the GG (40%); 0.25 in the AG (35%) and 0 in the AA
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49 176 genotype (15%). The calculated sample size, in order to detect a clinically relevant threshold of
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51 177 0.25 units (0.5-0.25=0.25) in the primary outcome of PAQLQ score (SD=1.0; alpha 0.05; 90%
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54 178 power) was 100 participants in each group. To allow for a 15% attrition rate, the recruitment
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56 179 target was increased to 120 participants in each group.
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Analyses comparing personalized and standard care were completed as pre-specified in the statistical analysis plan. All analyses were performed on intention to treat population i.e. by group randomized. Data for continuous outcome measures were assessed for normality prior to analysis. Transformations of the outcome variables were used where necessary if they were not normally distributed. If data were normally distributed, outcome measures were assessed using mixed model repeated measure analysis, adjusted for the corresponding baseline values and group allocation as fixed effects. Models used all available data from the end of the run-in period. Where data was not normally distributed and could not be transformed into a normal distribution, data was analyzed using non-parametric methods. Subgroup analyses were performed on participants with the Arg16Gly status AA. A two-sided P value of <0.05 was taken to be significant for all analyses. SAS software (version 9.4, SAS Institute Inc. Cary, NC, USA) was used for all statistical analyses. PACT conforms to CONSORT 2010 guidelines on RCT reporting.

Results

Between February 9, 2016 and April 25, 2018, 247 participants were consented and 241 randomized before entering the 4-week run-in period (figure 1). Participants were randomized to either personalized care (n=121) or standard care (n=120) for their asthma controller therapy. Baseline demographics and clinical characteristics were broadly similar between those receiving personalized or standard care with a mean age of 14.7 years across the two groups (table 1). There are however important differences between the groups with a greater proportion of adolescents receiving ICS, LABA and LTRA combination therapy at baseline in the standard care group (15% vs 6.6%) indicating a possible increased asthma severity in this group, and a lower prevalence of the AA genotype in the personalized care group (9.9% vs

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2 206 15.0%), possibly impairing the overall clinical benefit of personalized medication prescribing in
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5 207 this group.
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9 209 Completion of the run-in period (ICS only) resulted in 0.1 mean improvement in PAQLQ
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11 compared to baseline in both groups. A mean improvement in PAQLQ total score compared to
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13 end of run-in was observed in both the personalized and standard care groups (table 2).
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18 213 Prescription of asthma controller medication as per Arg16Gly genotype SNP status
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20 214 (personalized care) resulted in an improvement in mean PAQLQ compared to standard care
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22 (0.16, 95% CI 0.00-0.31; p=0.049; table 2; figure 2a; figure 3) however, the difference was
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24 below the pre-determined clinical threshold of 0.25. The PAQLQ domains of emotional
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26 function and activity limitation score had the greatest mean difference in change with
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28 personalized care.
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33 220 Sub-group analysis of the children with the homozygous AA genotype (n=27) demonstrated an
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35 improvement in mean PAQLQ that exceeded the clinical threshold of 0.25 in those receiving
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37 personalized compared to standard care (0.42, 95% CI 0.02-0.813; p=0.04; table 2; figure 2b).
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40 223 There were no adverse or serious adverse events reported throughout the duration of the trial.
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44 225 A total of 28 (11.6%) participants experienced an asthma exacerbation (requirement for oral
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46 steroids) during 12-month follow-up, with numerically lower rates reported in the personalized
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48 care (8.3%) compared to standard care group (15.3%) (p=0.08). There was also a trend toward
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50 increased time to exacerbation in the personalized care (225.7 days) compared to the standard
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52 care group (141.5 days) (p=0.102). There was a similar improvement in ACQ score from the end
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54 of run-in when mean change was compared in the personalized care (0.42) and standard care
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groups (0.47) ($p=0.18$). There was no association between personalized asthma controller directed prescribing and health care utilization or the number of asthma medications prescribed with a small increase in the mean number prescribed in the personalized care (0.3) and standard care groups (0.2) ($p=0.36$) (table 3).

Discussion

The PACT study is the first prospective RCT assessing the efficacy of genotype-directed prescribing in adolescent asthma. Prescription of second line controller medication with LABA or LTRA according to Arg16Gly genotype resulted in a statistically significant improvement in primary clinical outcome of PAQLQ, although the magnitude of improvement amounted to 0.16 and was below the a priori clinical threshold of 0.25. The quality of life benefit seen in adolescents with the homozygous AA genotype was 0.42, exceeding the clinically significant PAQLQ threshold.

Our findings are consistent with the only previous trial conducted examining the effects of LABA in relation to Arg16Gly genotype in children. That proof-of-concept study focused on previously genotyped asthmatic children who were all homozygous for the AA variant. A total of 62 children were randomized to receive either ICS and LABA or ICS and LTRA and were followed-up for 12-months. Children treated with a LTRA compared to LABA had a clinically relevant mean improvement in PAQLQ scores amounting to 0.53 as well as reduced school absences and use of rescue medication in comparison to those treated with LABA. [14] In keeping with these findings, adolescents with the AA homozygous genotype benefitted most from genotype-directed prescribing in our current study.

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2 255 Both RCTs are underpinned by a large meta-analysis of observational studies comprising five
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5 256 childhood asthma cohorts demonstrating an increased risk of exacerbations with each copy of
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7 257 the A allele amounting to a 34% (95% CI 15-50) difference for asthmatic children treated with
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10 258 ICS-LABA therapy [7]. In this study, we report a non-significant numerical trend toward
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12 259 decreased exacerbation frequency in the genotype directed treatment group (8.3% vs. 15.3%,
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15 260 p=0.08). The low exacerbation frequency (11.6%) within our well-controlled population
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17 261 recruited for this study is a likely confounding factor as to why this well described association
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20 262 did not reach significance. A 2018 systematic review further assessed the role of Arg16Gly
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22 263 genotype variation on LABA response in asthma, confirming that the contribution of genetics
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24 264 to LABA response is more consistently shown in children compared to adults [20]. It is
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27 265 hypothesized that this could relate to the altered phenotype of children's asthma with a
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29 266 greater emphasis of atopy in its pathogenesis as well as reduced duration of chronic airway
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32 267 inflammation and airway wall rigidity [21]. It is thus possible that a greater mean improvement
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34 268 in PAQLQ could be achieved with this genotype-directed intervention in younger children,
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37 269 especially in those with more severe disease.

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42 271 A conspicuous observation from the study was that both the personalized and standard care
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44 272 groups had improved PAQLQ and ACQ at final follow-up, this notable 'trial effect' is well
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47 273 described [22]. A component of this known as the 'care effect' could result from participants in
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49 274 both arms of the PACT trial having more regular face-to-face contact with primary care
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52 275 healthcare practitioners. The frequent telephone or e-mail communication with the study
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54 276 team as per the study protocol may have altered health behavior - the Hawthorne effect [23].
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56 277 It is also conceivable that by virtue of regularly completing the PAQLQ and ACQ questionnaires
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59 278 adolescents may have developed improved asthma symptom awareness, promoting self-
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279 education about asthma, triggers and the benefits of controller treatment and improved

adherence. This, potential 'trial effect', may explain how the mean improvement in PAQLQ exceeded the MCID in both groups which may in-turn have contributed to a smaller than anticipated response to genotype-directed therapy.

Our study has some limitations. The lower than expected effect of personalized medicine on PAQLQ score could partly be explained by the excellent symptom control at baseline with very good PAQLQ and ACQ scores demonstrated, leaving limited room for further improvement. This explanation is supported by the large number of adolescents who did not experience an exacerbation (88.4%) and did not require any non-routine healthcare utilization for their asthma (71.4%) during 12-month follow-up. The good baseline control on ICS treatment following the run-in period resulted in 65% of adolescents in the personalized medicine group not actually experiencing specific genotype based prescribing e.g. add on of LABA or LTRA thus diminishing the possibility of the benefit of personalized begin proven. Future studies aiming to identify the potential role of Arg16Gly genotype directed therapy may require selective recruitment of children with poorly controlled asthma. Differences between the groups could also explain the lower than anticipated response to personalized treatment. The greater proportion of adolescents receiving ICS, LABA and LTRA combination therapy at enrolment in the standard care group (15% vs 6.6%) could indicate potentially increased disease severity at baseline. A further contributing factor could be the lower than anticipated number of adolescents with the significant AA genotype in the personalized medicine group (9.9%) compared to the standard care group (15%) and a previous large population meta-analysis (16.1%) [7].

Ideally, the criteria for asthma diagnosis should be defined in accordance with the Global Initiative for Asthma (GINA) guidelines and documented for each patient involved in the study.

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2 305 By recruiting patients with a physician diagnosis of asthma from primary care, where there
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5 306 may not be routine access to spiromery or the capability to perform bronchodilator
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7 307 reversibility, the accuracy of asthma diagnosis is impaired which is a notable limitation.
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10 308 However, as the vast majority of adolescents with asthma are managed in this setting this
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12 309 approach assesses the pragmatic real world efficacy of personalised asthma prescribing. The
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14 310 lack of ethnic diversity within the study participants (88.8% Caucasian) is a potential limitation.
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17 311 There is however, reason to believe our results could be generalizable to other populations
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19 312 given the finding of increased rate of exacerbation with LABA use with presence of the A allele
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22 313 amongst cohorts of young people of differing ethnicities [7].
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26 315 An important element of the PACT study is in relation to the novel study methodology
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28 316 employed, utilizing telephone and online contact for consent and data collection along with
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31 317 study directed prescribing through the patients' GP without direct face-to-face contact with
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34 318 study team members at any point. This is unique in the investigation of young people's asthma.
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36 319 Replication of this methodology may help investigators to conduct trials in a safe manner
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39 320 during the Covid-19 pandemic. The significant associated cost reduction has important
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41 321 implications for future research studies, with the cost per patient of \$1495 for the PACT study
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44 322 substantially lower compared to a mean cost of \$4630 per patient for RCT's funded by the
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46 323 NIHR (National Institute for Health Research) Health Technology Assessment (HTA) program
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49 324 between 2000-2005 [24].
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54 326 Further areas need to be explored more fully to understand the potential value of Arg16Gly
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56 327 genotype directed prescribing in the treatment of children's asthma especially those with more
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59 328 severe disease. On the basis of our findings it is important to re-evaluate our study question in
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329 a less well-controlled sample including younger children with suggestions that the benefits of

Arg16Gly genotype based prescribing are more pronounced in this group. It is hoped that we will have a greater understanding following publication of the PUFFIN trial, an in progress multi-center Dutch study exploring Arg16Gly genotype based prescribing in 6-17 year-olds with asthma utilizing asthma control test as the primary outcome measure [25].

Conclusion

In this 12-month trial, asthma controller prescribing on the basis of Arg16Gly beta-2 receptor genotype resulted in a small but significant improvement in PAQLQ in adolescents compared to standard care, however this was below the expected clinical threshold. A clear benefit was demonstrated for those with the AA homozygous genotype (15% of the population). We recommend further prospective randomized studies to help identify the potential clinical utility of personalized prescribing in young people's asthma.

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis an interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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Figure legends

Figure 1 - Trial profile

Figure 2a - Pediatric asthma quality of life questionnaire total score

Data represented includes the median, 25th centile, 75th centile, lowest value and highest value. PAQLQ – Pediatric Asthma Quality of Life Questionnaire.

Figure 2b - Pediatric asthma quality of life questionnaire total score for A/A genotype

Data represented includes the median, 25th centile, 75th centile, lowest value and highest value. PAQLQ – Pediatric Asthma Quality of Life Questionnaire.

Figure 3 - Pediatric Asthma quality of life questionnaire total score change from baseline to 12 months follow-up

MCID – minimum clinically important difference

Table 1 – Baseline demographics and clinical characteristics

	Personalized care N=121	Standard care N=120
Age, years	14.6 (1.95)	14.7 (1.87)
Sex		
Male	57 (47.1%)	52 (43.3%)
Female	64 (52.9%)	68 (56.7%)
Genotype		
GLY/GLY (G/G)	53 (43.8%)	48 (40%)
ARG/GLY (A/G)	54 (44.6%)	53 (44.2%)
ARG/ARG (A/A)	12 (9.9%)	18 (15%)
Missing*	2 (1.7%)	1 (0.8%)
Ethnicity		
White	111 (91.7%)	103 (85.8%)
Black	0 (0%)	3 (2.5%)
Asian	1 (0.8%)	3 (2.5%)
Other	9 (7.4%)	11 (9.2%)
Baseline medications		
ICS	78 (64.5%)	79 (65.8%)
ICS + LABA	30 (24.8%)	19 (15.8%)
ICS + LTRA	5 (4.1%)	4 (3.3%)
ICS + LABA + LTRA	8 (6.6%)	18 (15%)
Healthcare use in previous month	14 (11.6%)	16 (13.3%)

* Participants withdrew from the study without providing saliva specimen

Data are mean (SD) or n (%)

ICS - Inhaled corticosteroid, LABA - Long-acting β 2-agonist, LTRA - leukotriene receptor antagonist

Table 2 – Pediatric asthma quality of life questionnaire (PAQLQ) score change

	End of run-in values	12 month values	Mean change	Overall mean difference in change *	
	Personalized Standard	Personalized Standard	Personalized Standard	95% CI	P value
PAQLQ total score	5.97 (5.80-6.14)	6.44 (6.31-6.57)	0.45 (0.27-0.63)	0.16 (0.00-0.31)	0.049
PAQLQ emotional function score	5.91 (5.71-6.10)	6.44 (6.32-6.56)	0.53 (0.35-0.72)	0.23 (0.04-0.42)	0.02
PAQLQ environment stimuli score	5.88 (5.65-6.10)	6.42 (6.25-6.59)	0.50 (0.26-0.74)	0.12 (-0.08-0.32)	0.25
PAQLQ activity limitation score	5.82 (5.60-6.04)	6.35 (6.18-6.52)	0.49 (0.26-0.73)	0.14 (0.00-0.28)	0.04
PAQLQ symptom score	6.21 (6.07-6.35)	6.60 (6.49-6.71)	0.36 (0.21-0.51)	0.16 (-0.02-0.33)	0.08
PAQLQ total score (A/A)	6.08 (5.91-6.24)	6.50 (6.38-6.61)	0.41 (0.25-0.57)	0.42 (0.02-0.81)	0.04 **
	5.81 (5.62-6.01)	6.30 (6.13-6.47)	0.48 (0.25-0.72)		
	5.67 (5.46- 5.89)	6.19 (6.03-6.34)	0.48 (0.24-0.71)		
	5.59 (4.99-6.20)	6.54 (6.12-7.00)	0.92 (0.4-1.44)		
	5.77 (5.12-6.42)	6.36 (6.14-6.59)	0.64 (0.06-1.22)		

Data are mean and 95% CI unless otherwise indicated.

PAQLQ – standardized pediatric asthma quality of life questionnaire.

* adjusted for treatment, genotype and baseline values.

** adjusted for treatment, genotype, baseline values and SAP characteristics (sex, age, site and

Index for Multiple Deprivation (IMD))

Table 3 – Secondary outcomes measures

	Personalized care	Standard care	Overall mean difference in change*	
			Hazard Ratio (95% CI)	p-value
Any exacerbation, number (%)	10 (8.3%)	18 (15.0%)	0.52 (0.24-1.36)	0.10
Time to first exacerbation, days	225.7 (121.3-330.1)	141.5 (78.7-204.3)	2.11 (0.91-4.87)	0.08
No health care utilization, number (%)	87 (71.9%)	85 (70.8%)	0.98 (0.56-1.72)	0.73
Number of medications, mean change	0.3 (0.15-0.42)	0.2 (0.07-0.29)	1.71 (0.85-3.45)	0.36
ACQ-6, mean change	-0.42 (-0.60-0.42)	-0.47 (-0.66-0.27)	-0.10 (-0.25-0.05)	0.18

Data are mean (SD) or n (%).

PAQLQ – standardized pediatric asthma quality of life questionnaire.

AQoL Total Score

Treatment allocation ●●● Personalized Care (Arg homozygous) ●●● Standard Care (Arg homozygous)

News release

Embargo: 00:01 hrs CEST, Tuesday 8 September 2020

NB: This presentation will be the subject of an embargoed news briefing by Dr Tom Ruffles at 11.00 hrs CEST on Friday 4 September

First trial of personalised care for children with asthma suggests benefits of prescribing according to genetic differences

Selecting treatments according to genetic differences could help children and teenagers with asthma, according to research presented at the European Respiratory Society International Congress. [1]

The trial, which compares patients treated according to small genetic differences with patients treated according to existing guidelines, is the first of its kind in children and teenagers.

Researchers say that more work is needed, but their findings hint that children's asthma symptoms could be better controlled with personalised treatments.

The study was presented at the virtual conference by Dr Tom Ruffles, honorary consultant in paediatric respiratory medicine, who worked with the study team led by Professor Somnath Mukhopadhyay, Chair in Paediatrics. Both are at the Royal Alexandra Children's Hospital, Brighton & Sussex Medical School, UK. The trial was conducted and managed by the Tayside Clinical Trials Unit at the University of Dundee, UK.

Dr Ruffles told the conference: "Asthma is a common condition in children that causes coughing, wheezing and difficulty breathing. In the UK, for example, asthma affects one in 11 children and every 18 minutes a child is admitted to hospital because of their asthma.

"We have a number of medicines that are generally effective in treating children with asthma, but they don't work equally well for all children. We think that genetic differences could have an effect on whether these medicines work, and that's what we wanted to examine in this study."

The research involved 241 children aged between 12 and 18 years who were all being treated for asthma by their GPs. The children were randomly assigned either to receive treatment according to existing guidelines, or to receive treatment according to particular genetic differences (their genotype), an approach known as personalised medicine.

Treatment according to genotype meant the children were asked to give a sample of cells scraped from the inside of their cheeks. These samples were tested for different versions of a particular gene, using a test that costs less than €20.

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The researchers were testing for a small difference in the gene that contains instructions for making the beta-2 receptor and they were looking for children who had either with one copy of the altered gene or with two copies of the altered gene. The beta-2 receptor is the molecule that is targeted by asthma treatments. Therefore, researchers believe that different versions of the gene for making this receptor can influence how well treatments work.

Previous research suggests that the majority of children with asthma will benefit from standard treatment with an asthma preventer called salmeterol in addition to their regular steroid inhaler. However, around one in seven children have a small genetic difference that means using this medication could actually result in these children having more asthma symptoms.

In this trial, children in the personalised medicine group who had this genetic difference were treated with an alternative asthma medicine called montelukast.

Researchers followed the children for a year to monitor their quality of life, with a score between one and seven according to how their symptoms were, whether their normal activities were limited by their asthma and how their asthma made them feel.

They compared the average score of the group of children who had their medication decided on the basis of their genetics with the average score of children who were treated according to current practice that does not involve any genetic testing, and found only a small improvement of 0.16 with those who received personalised care.

However, when the researchers looked specifically at the children who were found to have two copies of the altered beta-2 receptor gene, they found a greater benefit, with children experiencing an average 0.42 improvement in their quality of life score. The researchers say this would translate to a noticeably better quality of life for the children with two altered gene copies.

Professor Mukhopadhyay said: “These results are very promising because they show, for the first time, that it could be beneficial to test for certain genetic differences in children with asthma and select medication according to those differences.

“In this study, we saw only a modest effect, but this may be partly because the children’s asthma was generally very well controlled and only a few children experienced any serious symptoms during the 12-month period. Larger trials, with a focus on those with poorer asthma control, may help us determine the true benefit for children of prescribing in this way.”

Professor Chris Brightling, from the University of Leicester, UK, is European Respiratory Society Science Council Chair and was not involved in the research. He said: “This approach of ‘personalised care’ for both children and adults with asthma is an important goal for respiratory research.

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“In this study, researchers used genetic information that we know is linked to how well patients respond to some inhaler treatments. They found that making use of this genetic information improved the outcome for children with asthma.”

(ends)

Notes to editors

[1] Abstract no: 4617 “Effect of controller prescribing according to rs1042713 genotype on asthma related quality of life in young people (PACT): a randomized controlled trial”, by Tom Ruffles *et al*; Presented in “ALERT: Asthma in adults and children” 14:30-15:30 hrs CEST, Tuesday 8 September 2020. Dr Ruffles presentation is at 15:02 hrs CEST:

<https://k4.ersnet.org/prod/v2/Front/Program/Session?e=259&session=12681>

Funding: The study was funded by The Henry Smith charity and Action Medical Research (grant number GN2203).

Note: When obtaining outside comment, journalists are requested to ensure that their contacts are aware of the embargo on this release.

The European Respiratory Society (ERS) International Congress is the once-a-year occasion when the world's respiratory experts meet to present and discuss the latest research on topics such as asthma, COPD, lung cancer, pollution and smoking. Due to coronavirus, the Congress is going virtual for the first time ever, with over 25,000 participants from all over the world taking part in the exchange of scientific and clinical excellence across the entire field of respiratory medicine. The reputation of ERS derives from the outstanding scientific programme of its International Congress, which is now the largest respiratory meeting in the world.

<https://erscongress.org>

Further information:

CONSORT CHECKLIST

Table. CONSORT 2010 Checklist of Information to Include When Reporting a Randomized Trial^a

Section and Topic	Item No.	Checklist Item	Reported on Page No.
Title and abstract	1a	Identification as a randomized trial in the title	
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	
Introduction Background and objectives	2a	Scientific background and explanation of rationale	
	2b	Specific objectives or hypotheses	
Methods Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined prespecified primary and secondary outcome measures, including how and when they were assessed	
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomization Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomization; details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome	
	13b	For each group, losses and exclusions after randomization, together with reasons	
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analyzed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing prespecified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Comment Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	
	21	Generalizability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
Other information Registration	23	Registration number and name of trial registry	
	24	Where the full trial protocol can be accessed, if available	
	25	Sources of funding and other support (such as supply of drugs), role of funders	

^aWe strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomized trials, noninferiority and equivalence trials, nonpharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up-to-date references relevant to this checklist, see <http://www.consort-statement.org>.

Online Data Supplement

Asthma prescribing according to Arg16Gly beta-2 genotype: a randomized trial in adolescents

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Inclusion and Exclusion Criteria

Inclusion criteria

- a. Parent/Guardian/Participant is willing and able to give informed consent/assent
- b. Physician-diagnosed asthma
- c. Aged 12-18
- d. Taking inhaled corticosteroids (ICS) with/without second line controllers (i.e. LABA and/or LTRA)

Exclusion criteria

- a. Participant/Parent/Guardian is unwilling or unable to give informed consent/assent
- b. Participant has to be assessed as competent to provide consent.
- c. Known contraindication to montelukast or salmeterol
- d. On step 4 asthma control medication e.g. taking Theophylline, Slo-phylin, Uniphyllin

- e. Other major airway or lung disease, e.g. chronic lung disease of prematurity, cystic fibrosis, and abnormal airway anatomy
- f. Pregnant or lactating females (if participants become pregnant during the course of the study they will be asked to inform the research team and be withdrawn from the study)
- g. Participating in another clinical trial (other than observational trials and registries) concurrently or within 30 days prior to screening for entry into this study
- h. Unable to provide saliva/buccal cells for genotyping

Study Protocol

Does the Prescribing of Asthma Controller Medication According to Beta2 Receptor Gene Status Improve Quality of Life in 12-18 year olds with Asthma?

Study Acronym	PACT - Personalised medicine for Asthma ConTrol
Sponsor	University of Sussex
Sponsor R&D Number	14/054/MUK
Funder	Action Medical Research
Chief Investigator	Professor Somnath Mukhopadhyay
REC Number	15-ES-0007
IRAS ID	164449
ISRCTN/ClinicalTrials.gov Number	NCT02758873
Version Number and Date	Version 6 31.03.17

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Substantial Amendment No.	Protocol Version No.	Protocol Date	Author(s) of changes	Details of Changes made
1	4	25-04-15	Professor Somnath Mukhopadhyay/ Dr Roberta Littleford & Dr Christina Jones	Replace Paediatric Asthma Quality of Life Questionnaire (PAQLQ) with Standardised Asthma Quality of Life Questionnaire (AQLQ(S)). Addition of token of appreciation for participants. Notification to secondary care

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				physicians of participant's participation and genotype.
3	5	22-02-16	Professor Somnath Mukhopadhyay/ Dr Roberta Littleford & Kristina Pilvinyte	Clarification of assent/consent procedures. Additional inclusion and exclusion criteria. Clarification of saliva test and results pathway. Change of ACQ cut-off levels to < 1 and ≥1. Removal of incorporated ICF from PILs. Amendment to initial invitation pack contents.
4	6	31-03-17	Professor Somnath Mukhopadhyay/ Dr Roberta Littleford, Dr Christina Jones & Kristina Pilvinyte	Additional exclusion criteria. Addition of targeted social media as recruitment option. Removal of Manchester as secondary care site. Removal of secondary care clinic study visits. Addition of PIC sites. Addition of Optional Study Evaluation Questionnaire and Interview. Amended saliva sample collection processes.

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LIST OF ABBREVIATIONS

ACQ	Asthma Control Questionnaire
AE	Adverse Event
AQoL	Asthma quality of life
ARG	Arginine
BTS	British Thoracic Society
CI	Chief Investigator
CNORIS	Clinical Negligence and Other Risks Scheme
CRF	Case Report Form
CTM	Clinical Trial Manager
GCP	Good Clinical Practice
GLY	Glycine
GP	General Practitioner
HIC	Health Informatics Centre
HRA	Health Research Authority
IC	Inclusion Criteria
ICF	Informed Consent Form
ICH	International Conference of Harmonisation
ICS	Inhaled Corticosteroids
ISF	Investigator Site File
ITT	Intention to Treat
LABA	Long-acting β 2agonists
LPLV	Last Participant Last Visit
LTRA	Leukotriene Receptor Antagonists
NHS	National Health Service
NCTIMP	Non- Clinical Trial of Investigational Product
PI	Principal Investigator
PIS	Participant/Participant Information Sheet
REC	Research Ethics Committee
RN	Research Nurse
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
TCTU	Tayside Clinical Trials Unit
TMF	Trial Master File
TMG	Trial Management Group

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SUMMARY

LAY SUMMARY

One in every 11 children in the UK has asthma. Children with asthma cough, wheeze and have difficulty breathing. The symptoms which children experience can mean they miss school and makes it difficult for children to take part in playground games and sports. Some have to be admitted to hospital. In fact, in the UK a child is admitted to hospital every 18 minutes because of their asthma.

Effective medicines are available, but a child's response to these medicines is currently unpredictable. This project focuses on an asthma controller medicine called salmeterol. According to reports, tens of thousands of children may be taking this medicine in the UK, but evidence suggests it might not work for around one in seven of them. We are investigating whether a new approach to treatment, where prescribing is personalised according to a child's genetic make-up, improves the child's quality of life and provides better control of their asthma. Treatment that is tailored in this way to a person's genetic features is often called 'personalised medicine'.

At the moment, doctors commonly prescribe salmeterol to relieve asthma symptoms if children do not benefit enough from other medicines. But evidence suggests salmeterol may not work properly in children with a certain genetic makeup.

We are investigating whether it helps to take children and young people's genetic makeup into account when deciding whether to give them salmeterol or an alternative medicine called montelukast. A simple and inexpensive saliva test can provide the information needed to guide decision making.

1 INTRODUCTION

1.1 BACKGROUND

Asthma is a common chronic illness in children and young people [1]. It affects, for example, an average of two children in every UK classroom [2]. Initial treatment usually consists of salbutamol used on demand at step 1 of British Thoracic Society (BTS) guidelines [3]. At step 2, regular anti-inflammatory 'controller' therapy starts with the regular use of inhaled corticosteroids such as beclomethasone. Therapeutic efficacy with inhaled steroids usually peaks around 400 micrograms per day of beclomethasone (or equivalent). With inadequate asthma control at step 2, inhaled long-acting β_2 agonists (LABA) such as salmeterol, or leukotriene receptor antagonists (LTRA) such as montelukast are added; this represents BTS step 3 for asthma management.

The improvement of asthma-related quality-of-life represents an important goal for the overall pharmacological management of asthma [4]. Juniper has developed and validated questionnaires for the measurement of asthma-related quality-of-life and asthma control in both children and adults [4]. Furthermore, Juniper has defined the minimum improvement in asthma-related quality-of-life that is clinically relevant for participants with this disease [5]. In addition, Juniper has recently validated on-line versions of the asthma-related quality-of-life and asthma control questionnaires in children aged 12 and above [6]. This could provide an opportunity for more long-term and cost-effective comparisons of different asthma treatments through the use of tools that measure asthma control without the need for clinic visits and the completion of paper-based forms.

Overall, in children with asthma managed on step 3, salmeterol appears to provide better asthma control than montelukast in the setting of a randomized controlled trial [7]. However, in real life, the efficacy of salmeterol at step 3 for improving asthma control in individual children appears rather variable, and some children continue to experience day-to-day symptoms and exacerbations [8,9].

In CI's previous study of 1182 UK children and young adults (4-22 years), 50% of those on regular salmeterol experienced asthma exacerbations over a 6-month period, and 18% required inhaled salbutamol at least daily for symptom relief. Indeed, we reported a step-wise increase in the risk of asthma attacks related to each copy of the Arg16 allele on the β_2 receptor gene (1.7-fold) in asthmatic children and young adults exposed to regular salmeterol in conjunction with inhaled corticosteroids [8,9]. This led us to hypothesize that, contrary to the observations on the overall population of children and young adults where salmeterol is superior in efficacy to montelukast at step 3 [10], those possessing susceptible Arg16 β_2 receptor genotype may experience better asthma control with the addition of montelukast rather than salmeterol as second-line controller medication, in addition to inhaled corticosteroids. As such we elected to identify from our database those children with two copies of the Arg16 polymorphism [i.e. homozygous Arg genotype (~15% of overall population) who would potentially be at greatest risk]. The mechanism for worse control with regular salmeterol involves a greater susceptibility to agonist-induced down-regulation and uncoupling of airway β_2 receptors and associated sub-sensitivity of response in the Arg16 genotype [11].

We therefore performed a proof-of-concept randomized controlled trial to determine whether genetically susceptible children with homozygous Arg16 genotype experience superior long-term asthma control with montelukast compared with salmeterol when used as tailored second-line controller therapy as add-on to the inhaled steroid fluticasone. The purpose of this preliminary study was to provide evidence to support the potential for personalised

1 medicine based on the individual genotype to improve asthma-related quality-of-life and
2 control. This study was published in 2013, and represents the first prospective randomized
3 controlled study in children with asthma that addresses personalised medicine based on
4 genotype. The results of this study showed that in children expressing the homozygous Arg
5 16 genotype, in comparison with salmeterol, adding montelukast to inhaled fluticasone
6 significantly improved asthma-related quality-of-life and clinical symptoms, while reducing
7 school absences and inhaled reliever use. The relative benefits of montelukast in comparison
8 with salmeterol became evident within the first 3 months and persisted throughout the whole
9 year [12].

13 Subsequently, we used Pubmed to search the Medline database for other randomised
14 controlled trials comparing the effects of salmeterol (or other long-acting beta2 agonist) with
15 montelukast (or other leukotriene antagonist) within the context of Arg/Gly variation, in
16 children with asthma. No studies could be identified. In particular, there are no trials in either
17 adults or children that have studied quality-of-life, which is a key outcome of interest in the
18 context of asthma-related disability, and which is often unrelated to outcomes such as lung
19 function. The absence of other randomised trials, together with the strength of our proof-of-
20 concept evidence [12], underscores the need for this powered study.

24 **1.2 RATIONALE FOR STUDY**

26 Children with asthma inadequately controlled on inhaled steroids as ‘controller’ medication
27 experience greater benefit in asthma-related quality-of-life from allocation of further drug
28 therapy on the basis of their genetic status, in comparison to allocation on the basis of
29 current method of doctor or nurse choice informed by the BTS guidelines.

32 **2 STUDY OBJECTIVES**

34 **2.1 OBJECTIVES**

36 **2.1.1 Primary Objective**

37 Does the prescribing of asthma controller medication according to beta2 receptor gene
38 status improve quality of life as determined by validated questionnaire (Juniper) in 12-18 year
39 olds with asthma?

42 **2.1.2 Secondary Objectives**

43 Asthma control, as determined by validated questionnaire at 3, 6, 9 and 12 months and an
44 evaluation of how many visits participants report to have attended their GP or asthma nurse
45 (non- routine asthma review), A&E or been hospitalised as a result of their asthma. Courses
46 of oral steroids and any other medication taken will also be recorded.

49 **2.2 OUTCOMES**

51 **2.2.1 Primary Outcomes**

- 52 ➤ Minimum clinically relevant difference (0.25 units) in the asthma quality of life (AQoL)
53 score.

56 **2.2.2 Secondary Outcomes**

- 57 ➤ Asthma control measured by the asthma control questionnaire in addition to health
58 care utilisation (GP/asthma nurse visits outside of routine review, A&E attendances,
59 hospital admissions and use of additional medication.

3 STUDY DESIGN

PACT is a two-arm, multi-centre, randomised controlled trial designed to compare a tailored therapy intervention based on genetic status against standard participant asthma management regimes (BTS). The genetic group with Arg/Arg and Arg/Gly status will be recommended montelukast and children and young adults with Gly/Gly status will be recommended salmeterol.

The intervention period and follow up will last approximately 13 months per participant (4 week run-in plus 12 month follow-up assessments plus/minus visit windows). Outcomes will be measured at baseline, post run-in, and at 3, 6, 9 and 12 months. Study visits will take place primarily online, but can take place via telephone, post and face to face at secondary care research sites where available. Participants will be recruited via asthma research databases BREATH & PAGES, primary and/or secondary care in Scotland and England.

3.1 PARTICIPANT PATHWAY

3.2 STUDY MATRIX

Assessment/Procedures							
	Pre-Screen	Baseline	Run-in Start	Run-in Ends 4 weeks (-3/+7 days)	Follow-up @ 3, 6, 9 & 12 months (± 4 weeks)	Withdrawal Visit (optional)	End of study (optional)
Initial Pre -Screen	X						
Informed Consent/Assent ¹		X	X	X	X	X	
Inclusion / Exclusion Criteria		X					
Demographics		X					
Asthma - History/Treatment		X					
Asthma UK Asthma Attack advice card			X				
Randomisation			X				
Spit Test (if required) ²		X					
GP Visit ³			X	X			
Asthma Control Questionnaire (ACQ)		X		X	X	X	
Asthma Quality of Life Questionnaire		X		X	X	X	
GP Algorithm Letter ⁴			X	X			
Health/Asthma Medication Report		X		X	X	X	
Study Evaluation Questionnaire/Telephone Interview							X

¹ Written/Online informed consent/assent to be taken prior to any study procedures. Thereafter verbal/online check of consent/assent prior to any subsequent visit.

² Those with known asthma genotype status (database recruitment) will not require a spit test. Those with no known genetic status and randomised to guideline asthma control will require a spit test but its analysis will be carried out during the follow-up period.

³ Participants/parents will be asked to make an appointment to see GPs to discuss asthma treatment (if required).

⁴ GPs & Secondary Care Physicians will receive a treatment algorithm dependent upon the group allocation and genotype status.

STUDY POPULATION

3.3 NUMBER OF PARTICIPANTS

Two hundred and forty male and females aged between 12 and 18 will be recruited, with 120 randomly allocated to each group.

3.4 INCLUSION CRITERIA

- a. Parent/Guardian/Participant is willing and able to give informed consent/assent
- b. Physician-diagnosed asthma
- c. Aged 12-18
- d. Taking inhaled corticosteroids (ICS) with/without second line controllers (i.e. LABA and/or LTRA)

3.5 EXCLUSION CRITERIA

- a. Participant/Parent/Guardian is unwilling or unable to give informed consent/assent
- b. Participant has to be assessed as competent to provide consent.
- c. Known contraindication to montelukast or salmeterol
- d. On step 4 asthma control medication e.g. taking Theophylline, Slo-phylin, Uniphyllin
- e. Other major airway or lung disease, e.g. chronic lung disease of prematurity, cystic fibrosis, and abnormal airway anatomy
- f. Pregnant or lactating females (if participants become pregnant during the course of the study they will be asked to inform the research team and be withdrawn from the study)
- g. Participating in another clinical trial (other than observational trials and registries) concurrently or within 30 days prior to screening for entry into this study
- h. Unable to provide saliva/buccal cells for genotyping

4 PARTICIPANT SELECTION AND ENROLMENT

4.1 IDENTIFYING PARTICIPANTS

Multiple recruitment strategies will be employed. All methods of recruitment will utilise letters of invitation and/or Participant Information Sheets which are age appropriate.

1. Potential participants will be invited using the established asthma clinical research databases BREATHE (MREC Ref: 08/S0501/48) and PAGES (REC Ref: 07/H0203/204). These databases were created by members of the research team and collaborators from previous clinical studies. Only those participants who have consented to be contacted for future research will be approached.
2. Primary care recruitment will be carried out with the assistance of the primary care research networks in Scotland (SPCRN) and England (CRN). Potential participants will be identified from electronic patient records by appropriate CRN/SPCRN or GP practice staff. Practice Manager/GP/asthma or research nurse will verify the lists before patients are invited to participate. In addition, GPs will use optional opportunistic recruitment, i.e. inform their patients about PACT study, and direct them to the study team.
3. Specific secondary care clinics will also be involved in recruitment whereby respiratory physicians will inform their patients of the study. All interested participants will be requested to contact the study team for further information.
4. Asthma interest groups (e.g. Asthma UK) will be approached to inform them of the clinical study. If agreed, a link to the PACT study website will be added to their website to allow for ease of access to study information.

5. Other recruitment strategies may be employed including: targeted social media, a media campaign involving local and national newspapers, radio and television. Supplementary study advertising and information via primary and secondary care, community centres, pharmacies, sports halls etc. will be presented including, posters, brief information sheets and banners.

6. A participant specific section of the study website (www.pactstudy.org.uk) containing age specific PISs and research staff contact details will be available for participants to view.

4.2 CONSENTING PARTICIPANTS/PARENTS

Informed consent and assent should protect the child's rights, well-being as well as their autonomy, and should be an ongoing process of information exchange. The study will follow the Children's Research Network Standard Operating procedures, HRA guidelines and the Nuffield Council on Bioethics: Children and Clinical Research: Ethical Issues (2015, <http://nuffieldbioethics.org/project/children-research/>) obtaining informed consent. In research studies where a person under the age of 16 is considered competent to give an informed view on the question of participating in a research study, they should be allowed, and facilitated to do so. All participants in this study will consent for themselves where they are capable of understanding the nature, purpose and likely outcome of the research. Competency is required in the study as participants will be required to complete 3 monthly follow-up questionnaires. Parental assent will also be sought as parental support may be required for participation in the research study and will act as further safe-guarding of the child/young person for those 15 years and younger. Participants aged 16-18 will consent independently without parental assent (see Table 1 below).

Table 1. Summary of Consent/ Assent in Scotland and England for Non-CTIMP.

Competent Young Person/Adult	Assent/Consent
Person <16y	Consent Form 12-15 Assent - Parent
Person ≥16y	Consent Form 16-18

This study is a Non-CTIMP studying licenced products within the prescribed guidelines in a group of participants that understand and manage their asthma on a daily basis.

Potential participants who have consented to be contact via the BREATHE (MREC Ref: 08/S0501/48) and PAGES (MREC Ref: 07/H0203/204) databases will be sent an invitation letter/email (as indicated by participant/parent preferred method of communication) from the lead clinician with a brief information sheet (bPIS) for the participant and one for parent/guardian as appropriate.

Potential participants recruited via their General Practitioner (via clinical research networks) will also be sent an invitation pack consisting of a brief information sheet(s) and an invitation letter pack.

All invitation packs will be sent via Health Informatics Centre (HIC) Services. HIC is a University of Dundee research support unit within the Tayside Medical Science Centre (TASC). All services provided by HIC are delivered within an ISO 270001 certified secure environment to ensure data is managed safely and in compliance with Data Protection legislation. The identified invitation lists will be stored securely within the NHS network. The

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invitation pack will consist of an invitation letter with a reply slip, brief information sheet/leaflet and pre-paid return envelope. The research team will not have access to this confidential invitation list. HIC will also send any reminders with the relevant PIS. Any participants/parents seeking additional information will be directed to the study website (www.pactstudy.org.uk) which contains the PISs and ICFs with research staff contact details available for participants/parents to view.

Participants can indicate their interest in the study by calling/e-mailing the study team or completing and returning the reply slip using the pre-paid envelope. Those interested will be sent age appropriate information sheet with a parental/guardian PIS (if required). HIC will upload all positive replies allowing the relevant study team access to the potential participant's contact details. These details are uploaded onto the secure web based Recruitment Tracker. The Cohort ID generated during the invitation stage will be used as one of the participant identifiers. For participants who have actively stated they do not wish to be contacted, their details will be not be uploaded and they will not receive any further communication. These details are not visible to the study team. If required, a maximum of two reminder letters will be sent to participants, the first approximately 4 weeks after the initial invite with the final reminder approximately 4 weeks after the initial reminder. The Recruitment Tracker will document each positive response and with consent participant's contact details allowing the study team to efficiently communicate with participants/parents, GPs and secondary care physicians.

For data management purposes, identifiable information, completed consent forms and anonymised study data will be securely stored in locked cupboards and password protected databases in the University of Dundee. Identifiable information will be stored separately from study data. Authorised members of the trial and data management team will have access to identifiable information to allow the management of study visits.

Other potential participants will receive the Brief Participant Information Sheet (bPIS) at their routine care NHS clinic appointment. A contact telephone number, study website address and an e-mail address for the study team will be included to give the participant the opportunity to discuss the study. These participants can indicate their interest in the study by calling/emailing the study team. Participants receiving the bPIS and who express an interest in the study will receive full PIS by e-mail or mail from the study co-ordinator. For participants identified by their respiratory physician and approached at the clinic, the research/clinic nurse/associate will provide the PIS and discuss the study.

Participants approached at clinic or who have received study information from their GP and have indicated interest by returning the reply slip, have e-mailed or called the study team will be invited to perform screening which can take place online, via telephone or face to face (F2F) in secondary care clinics where available. All participants will have been given at least 24 hours to consider their response.

Participants who independently contact the study team having seen study information i.e. poster or bPIS, will be sent the age appropriate invitation pack with Parental/Guardian information as required.

Consent/assent will be obtained primarily online or can be obtained verbally via telephone call with follow-up written consent. Written informed consent/assent will be obtained from the parent/child/young adult if the visit is conducted face to face at the participating secondary care site.

After receiving full PIS the parent/participant can discuss the study with the trial team on the telephone and if they are eligible the appropriate consent/assent forms will be completed online (as per online process). Alternatively consent/assent forms can be posted to them along with the pre-paid envelope.

The researcher will document the initial verbal consent procedure in the participant's research notes and/or the Recruitment Tracker. When the consent documentation is received by the study office the researcher will sign the consent/assent form noting the date which will be after the date documented by the parent/participant.

All participants/parents will have had at least one telephone call, when eligibility will be established.

If the participant/parent wish to proceed with the study and to carry out online consent the researcher will document the request on the PACT Portal which will trigger an e-mail to be sent to both participant/parent as appropriate. The e-mail will contain a link to the PACT Portal, username and password. There will be a prompt to change the password at the first log in. The study Cohort ID will be the identifier which will link participant and parent consent/assent. The consent/assent form will request participant/parent to type in their name. The date of completion will be pre-populated. If appropriate both participant/parent consent/assent have to be completed before full online consent is activated. Once the participant and parent consent/assents are matched the trial co-ordinator will receive an email to notify them that full consent has been obtained. The trial co-ordinator/delegate will update the recruitment tracker and print out the consent forms via the PACT portal and post to the participant's GP, parent/participant (if required) with copies filed in the ISF. The participant/parent can download/print the completed consent/assent forms.

If the participant does not have an independent email they will be able to set-up a user account with an independent password. The initial email will be sent to the parent and the parent would receive the reminders for questionnaire completion as per study matrix, but the participant can log on independently to complete the study forms.

Participants without an email account will be given the option of completing the study documentation using the paper validated versions which will be sent to the participant with a reply paid envelope. On receipt the trial staff will enter the data and the scores obtained will be captured in the database.

For participants who have completed face to face and verbal consenting procedures and wish to complete study questionnaires and health form online they will be sent an email with login credentials and following the processes as outlined above to enable completion of the online forms (see Figure 1, HIC PACT Portal pathway)

If the participant/parent has mislaid the invitation letter they will be asked three other forms of identification: Name, Date of Birth and Address, in order to check the Cohort ID via the Recruitment Tracker. The study team will co-ordinate the study run-in period with the participant. The participant will not start the run-in period prior to receiving the consent documentation and a follow-up phone call to the participant indicating the consent form(s) have been received.

All individuals taking informed consent/assent in person will have received training in Good Clinical Practice (GCP). Informed consent/assent will be explained to participants that they are under no obligation to enter the trial and that they can withdraw at any time.

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Participants and parents are informed that they will be able to consult the study team to discuss any issues but are informed to seek medical advice from their GP and/or NHS 24 as required.

If a participant’s ACQ score increases to ≥ 1 during study participation, collected at follow-up visits, the participant will be requested to make an appointment to see their GP to discuss symptom control as per the treatment algorithm. The study team will inform the GP of the participant’s exacerbation via telephone call and follow up by email/fax/letter [13].

If new safety information results in significant changes to the study risk–benefit assessment, the Protocol, PIS and/or consent/assent form they will be reviewed, updated and amended as necessary. All participants, including those already being treated, will be informed of the new information, given a copy of the revised consent form and asked to re-consent by either of the three available methods if they choose to continue in the study.

The study team will contact participants by email/call/SMS to remind them of a follow-up visit. This will be conducted to increase study retention.

Participants will receive a gift token to the value of £10 for participating in the study. This will be a gift sent at the end of their participation, either the end of study or early withdrawal.

Figure 1. HIC PACT Patient Flow

4.3 SCREENING FOR ELIGIBILITY

After informed consent/assent is obtained and eligibility by all the criteria (protocol sections 3.4 & 3.5 confirmed, study assessments as outlined in section 3.1 will be performed.

4.4 INELIGIBLE AND NON-RECRUITED PARTICIPANTS

The reason(s) for ineligibility will be explained to the participant/parent in person or via the telephone and any questions they have will be answered. If ineligibility is established during completing the baseline inclusion list, either ineligibility will be flagged at the time of completion, with notification to contact the study team if they have any further questions. They will be thanked for their participation in screening.

4.5 RANDOMISATION

4.5.1 Randomisation

Randomisation will be via a centrally controlled web-based, GCP-compliant randomisation system, run by Tayside Clinical Trials Unit (TCTU) called TRuST (Tayside Randomisation SysTem). The randomisation program, seed and allocation will be securely backed up and will employ disaster recovery plans as per the HIC University of Dundee Standard Operating Procedures. Participants will be allocated to one of two groups as per block randomisation, with no stratification or minimisation:

Group 1: Personalised Medicine will be prescribed controller medication based on genetic test, Arg/Arg or Arg/Gly – Montelukast (LTRA) or Gly/Gly –salmeterol (LABA).

Group 2: Standard Care will be prescribed controller medication based on current BTS guidelines.

Neither group is blinded to group allocation or prescribed medication.

4.5.2 Intervention allocation

Participants will be randomly allocated in a 1:1 ratio to treat as per genotype; personalised medicine or standard care.

4.6 RUN-IN PERIOD

After consent is received participants will require a 4 week run-in period (-3/+7 days). Participants will be provided with an advice card produced by Asthma UK on how to manage an asthma attack in case of an emergency. For those participants taking add-on controller medication they will be asked to stop and only use inhaled corticosteroids (ICS) and reliever medication to control their asthma during the run-in period. Depending upon the participants baseline treatment regime they may require to switch their combined medication ie. Seretide to Flixotide or Symbicort to Pulmicort (see Figure 2). The GP will receive a treatment algorithm to assist with run-in and post randomisation prescribing (see Figure 2).

For participants that have no known genotype they will be requested to provide a spit test during the run-in period (see Section 4.8).

4.7 MEASURES

It is hoped that the majority of the questionnaires will be completed online. To facilitate this, participants who were not set-up with a username and password account as part of the consenting process and have indicated that they would prefer to complete the study questionnaires and health questionnaire online, will be sent an email with username and password (same process as informed consent online above).

Email prompt will be sent to participant (and parent if applicable) when study questionnaires are due to be completed online at each time point. Maximum of two reminders will be sent prior to the end of the visit window to encourage compliance. If the participant has not completed the questionnaires within the visit window the visit will be assumed missed and they will be contacted for the next visit. If required the study questionnaires can be posted to the participant if they are experiencing technical difficulties.

4.7.1 Standardised Asthma Quality of Life Questionnaire (AQLQ(S))

The Standardised Asthma Quality of Life Questionnaire (AQLQ(S)) was developed by Juniper et al [4]. It measures the functional problems (physical, emotional and social) that are most troublesome to children and young adults with asthma. The AQLQ(S) has 32 questions in 3 domains (symptoms, activity limitation and emotional function) and are recorded on a 7-point scale (7 = not bothered at all and 1 = extremely bothered). The overall AQLQ(S) score is the mean of all 32 responses and the individual domain scores are the means of the items in those domains. The (AQLQ(S)) differs from the original Asthma Quality of Life Questionnaire (AQLQ) in that it has "standardised" 5 activities to replace the patient-specific ones in the original AQLQ. In the original AQLQ the "patient-specific" identified five activities which he/she did regularly and which were limited because of asthma. These five activities remained constant for this patient at each follow-up visit. This proved to be time consuming as patients activities changed over time especially in the child/young adult group. The Standardised Asthma Quality of Life Questionnaire (AQLQ(S)) has been fully validated, has strong measurement properties and a change in score greater than 0.5 on the 7-point scale can be considered clinically important. The AQLQ(S) is now the most extensively used of all the asthma quality of life questionnaires because of its strong measurement properties and ease of use in the study population age group.

4.7.2 Asthma Control Questionnaire

The Asthma Control Questionnaire (ACQ) was developed by Juniper et al [14] to measures both the adequacy of asthma control and change in asthma control, which occurs either spontaneously or as a result of treatment. The ACQ has 6 questions (the top scoring 5 symptoms, and daily rescue bronchodilator use). Patients are asked to recall how their asthma has been during the previous week and to respond to the symptom and bronchodilator use questions on a 7-point scale (0=no impairment, 6= maximum impairment). The questions are equally weighted and the ACQ score is the mean of the 6 questions and therefore between 0 (totally controlled) and 6 (severely uncontrolled). The ACQ has strong measurement properties and has been fully validated for use in both clinical practice and clinical trials. For clinical practice, clinical trials and epidemiological studies, the ACQ has strong discriminative and evaluative properties which means that it can detect small differences between patients with different levels of asthma control and it is very sensitive to within-patient change in asthma control over time. The ACQ has been fully validated for all children 6-17 years when the self-administered adult version is used by children 11 years and older.

4.7.3 Additional health questions

Participant/trial co-ordinator will record how many times they have had to see their GP or asthma nurse (outside of routine asthma review), been to A&E or been admitted to hospital as a result of their asthma. Courses of oral corticosteroids for asthma and any other

medication will also be recorded at the same quarterly assessments as the AQLQ(S) and ACQ.

4.7.4 Study Evaluation Questionnaire

Participants will be invited to complete an optional Study Evaluation Questionnaire after completing their final follow-up questionnaires. In addition, participants will be asked if they would agree to be contacted by the researcher to discuss the study in more detail via a convenient telephone call. The interview will follow the Evaluation Interview Guide. A formal power calculation is not required for the qualitative design of the Study Evaluation Questionnaire and Interview. Recruitment will continue until theoretical saturation defined as no new themes emerging after three consecutive interviews (this is usually achieved within 20-30 participants (8-12% of the study population). All evaluation data will be stored as per the project's data protection guidelines. The information gained will assist in future online trial design in this unique population.

All questionnaires will be reviewed by Dr Christina Jones (collaborator, chartered psychologist) and any participant exhibiting any anxiety, dissatisfaction or indicating their requirement for additional information will be contacted by the chief investigator or Dr Jones to provide any necessary reassurance and information. We do not anticipate that this questionnaire will identify any high risk anxiety issues.

4.8 DNA COLLECTION

For a number of participants invited via the BREATHE and PAGES, their Arg/Gly genotyping will be known. For those participants a repeat DNA test will not be required. Participants without known Arg/Gly genotyping will be requested to provide a saliva sample. The study team will send participants a letter with saliva sample kit, instructions and replied paid envelope. To obtain the sample participants will be encouraged to have a drink of water prior to providing the saliva sample. The drink prior to the procedure enhances the production of saliva. After a few minutes they will be requested to spit in the container directly, up to the mark. Participant/parents will be asked to inform the study staff if they are unable to perform the spit test at home. If they are unable to perform the spit test then a cheek swab will be obtained. From the study team's experience the majority of participants are able to perform the spit test.

Chain of custody will be monitored via the study co-ordinating centre.

The non-invasive Oragene® DNA collection kits (<http://www.dnagenotek.com>) or equivalent will be used for the collection of saliva for DNA extraction purpose. The kit provides a median DNA yield of about 110 micrograms. The DNA from saliva is stable in this kit for up to 5 years at room temperature. The stability is achieved with proprietary reagents that inactivate bacteria and nucleases in saliva and minimize chemical hydrolysis of DNA. The kits will be posted by the participants/parents to Tayside Clinical trials Unit using pre-paid envelope. PACT trial co-ordinator will register the receipt of the sample and will deliver it to the Medical Research Institute, University of Dundee to Professor Colin Palmer, Chair of Pharmacogenomics for DNA extraction and Arg/Gly analysis.

Study team will upload the results onto the Recruitment Tracker using a coded system.

Rarely, the saliva sample is inadequate to provide the genotype. In this event participants will receive a phone call from the trial co-ordinator with a follow-up letter outlining the need for a repeat sample.

For those participants randomised into the Personalised Medicine Group the genotype and ACQ score will be used to establish the correct treatment pathway outlined in the GP Treatment Letter and sent to the participant's GP. Secondary care physician will be informed of participant's involvement, their group allocation and given a copy of the treatment algorithm for their records. For those allocated into the personalised medicine group they will receive notification of their patient's genotype, while those allocated to standard care group will be informed of genotype at the end of the participant's involvement in the study; either at end of study or early withdrawal. Parent/guardians and participants will be informed via the PIL that secondary care physicians require to be informed to provide clinical care. With consent, the samples will be stored for a period of 15 years and used for ethically approved medical research.

Figure 2. Treatment Algorithm

PACT TREATMENT ALGORITHM

R_x = GP ACTIVITY REQUIRED

PACT Treatment Algorithm V1 31-03-2017

4.9 WITHDRAWAL PROCEDURES

Any clinician involved in the usual care of participant may withdraw the participant from the study using his/her clinical judgement.

In PISs we explain to the potential participant/parent that entry into the study is entirely voluntary and that their treatment and care will not be affected by their decision. It will also be explained that they can withdraw at any time. In the event of their withdrawal no further evaluations will be performed and no additional data will be collected. Any data collected before such withdrawal will be retained and used in the study analysis (with consent). Future use of the DNA sample (if obtained) will be requested but where this is declined the remaining sample will be destroyed. The participant will be invited to complete the ACQ, AQoL questionnaires and health report on study withdrawal as part of a study withdrawal visit.

If a female participant becomes pregnant during the study period they are requested to inform the study team and the participant will be withdrawn from the study. The withdrawal is not for study safety rationale as the participant would continue on asthma medication during their pregnancy as part of their routine clinical care, but as pregnancy will affect the Quality of Life outcome measures.

Although a participant is not obliged to give reason(s) for withdrawing consent prematurely, the CI/delegate will make a reasonable effort to ascertain the reason(s), while fully respecting the individual's rights. The rationale for withdrawal may assist researchers involved to improve design of future studies.

5 STUDY & SAFETY ASSESSMENTS

As the study does not employ an Investigational Medicinal Product, Adverse Events (AE) or Serious Adverse Events (SAE) will be recorded and reported as per Health Health Research Authority (HTA) and Sponsor's guidelines (University Collaboration Agreement) (see Table 2 below). *A serious adverse event (SAE) is defined as an untoward occurrence that:*

- (a) results in death;*
- (b) is life-threatening;*
- (c) requires hospitalisation or prolongation of existing hospitalisation;*
- (d) results in persistent or significant disability or incapacity;*
- (e) consists of a congenital anomaly or birth defect; or*
- (f) is otherwise considered medically significant by the investigator.*

An SAE occurring to a research participant will be reported to the main REC where in the opinion of the Chief Investigator the event was:

Related – that is, it resulted from administration of any of the research procedures, and

Unexpected – that is, the type of event is not listed in the protocol as an expected occurrence.

Due to the nature of asthma it is envisaged that participants may experience exacerbations requiring hospitalisation (AE and ward admission) and as such these will be recorded as part of the health questionnaire but not reported as a SAE as defined above.

Table 2. Serious Adverse Event Reporting in Non-CTIMP Study

	Who	When	How	To whom
SAE	Chief Investigator (CI) or sponsor	Within 7-14 days of the CI becoming aware of the event (in line with the Sponsor's Collaboration Agreement	SAE report form for non-CTIMPs available from HTA website	Main REC for the trial
Urgent safety measures	Chief Investigator (CI) or sponsor <i>Or exceptionally by local principal investigator (PI)</i>	(i) Immediately (ii) Within 3 days	(i) By telephone (ii) Notice in writing setting out the reasons for urgent safety measures and the plan for further action	Main REC for trial REC co-ordinator will acknowledge within 30days <i>If notified by PI, relevant local REC should also be informed</i>

6 DATA COLLECTION & MANAGEMENT

6.1 DATA COLLECTION

Data collection and entry will be completed using a variety of methods depending upon participant's preference for study visits. If the participant/parent attends the clinic for a study visit the demographic, asthma and medical history, adverse events and concomitant medication could either be collected via a paper Case Report Form (pCRF) with transcription of the data onto study specific eCRF/database developed by HIC, University of Dundee or the online versions of the questionnaires: Asthma Control Questionnaire (ACQ,n=6) and AQLQ(S) will be used for all scheduled online and face to face visits. Therefore whether a participant attends the clinic or chooses to complete the questionnaires at home, direct data entry will be used. Paper versions will be used for those not wishing to complete online or face to face, with study team performing data entry on receipt of the postal questionnaires.

The Delegation of Responsibilities Log will identify all trial personnel responsible for data collection, entry, handling and managing the database.

Where data is collected by the trial co-ordinator/delegate it will be directly entered to web based eCRF or recorded onto a pCRF with subsequent transcription to the eCRF/database. If data is recorded by the participant or parent/guardian it will be completed online. All electronic storage of non-identifiable data will be on a password protected device and/or database.

If required, participant's medical notes (GP and hospital) paper or electronic will act as source data for relevant past medical history, subsequent medical conditions, hospital admissions and diagnostic reports.

6.2 DATA MANAGEMENT SYSTEM

A data management system will be provided by HIC, University of Dundee. The data will be collected by participant completing demographic, health, AQoL and Asthma Control questionnaires. All data will be collected using unique participant ID.

7 STATISTICS AND DATA ANALYSIS

7.1 SAMPLE SIZE CALCULATION

The asthma quality of life (AQoL) data will be collected at 2 baselines (0 and 4 weeks) and 4 follow up points: 3 months, 6 months, 9 months and 1 year. A change of 0.5 units on the Juniper AQoL is considered clinically significant and we expect to see an improvement of 0.25 in Group 2. The improvement on the AQoL at one year is estimated to be 0.5 in the 40% of GLY/GLY, 0.15 in the 35% of ARG/GLY, and 0 in the 15% of ARG/ARG genotypes in Group 2. That is overall, the improvement at one year is approximately 0.25. While in Group 1, the improvement on the AQoL at one year is estimated to 0.5 regardless of genotype.

Therefore, in order to detect a minimum clinically relevant difference of 0.25 units ($0.5 - 0.25 = 0.25$) on the AQoL score ($SD=1$; $\alpha=0.05$; 90% power), 100 participants are needed in each group. To allow for a 15% attrition rate, 120 participants will be recruited from each group. The data will be analysed using mixed models with one between group factor (genotype guided vs non genotype guided) and 6 repeated measures (two baselines and 4 time points). The sample size calculations have been provided by Ms. Stephanie Goubet, statistician, NHS Clinical Investigation and Research Unit, Brighton and Sussex University Hospitals NHS Trust.

7.2 PROPOSED ANALYSES

The primary analyses will be conducted according to the principles of intention to treat (ITT) as outlined on the ICH E9 'Statistical Principles for Clinical Trials'.

Continuous variables will be summarised by the number of observations, number of missing values, mean, standard deviation (SD), median, inter-quartile range (IQR) and range. Summaries will be provided at baseline, at each subsequent time point and for the change from baseline by intervention group. Categorical variables will be summarised by the number of observations, number of missing values and number and percentage in each category. Summaries will be provided at baseline and at each subsequent time point.

Data analysis will follow the method used for the proof-of-concept study [14]. Statistical analyses by ITT will be performed using SPSS for Windows and Prism (GraphPad Software) or equivalent. Comparisons will be made by repeated measures ANOVA for longitudinal data measurements. Outcome variables based on daily symptoms and diary records will be averaged over all the days between clinic visits.

A statistical analysis plan (SAP) will be written prior to data lock and approved by the statistician and CI.

7.3 MISSING DATA

The extent of missing data will be explored in the outcomes especially the primary outcome. Patterns of missing data will be explored and predictors of missingness examined, especially if these vary by intervention. If necessary, multiple imputation will be utilised to impute missing data assuming the missingness mechanism is missing at random (MAR). However, mixed models have the useful property of using all available data without resorting to imputation where data is MAR. The assumption of MAR cannot be pre-specified or tested as it depends on whether and how data has become missing. Measures should be taken where possible to minimise the extent of missing data in the recording of outcomes so that missing data is less likely.

7.4 TRANSFER OF DATA

Participants will be informed of data storage and consent will be sought. Data will be collected either via face to face clinic visits, by telephone consultation which will be documented by the participant and research team. The data will either be collected via a pCRF or directly onto the eCRF. All pCRFs will be anonymised using a unique study identifier and stored in a lockable filing cabinet within a locked, restricted access study site office. All data entered onto the study databases will be password protected using secure servers with access restricted to relevant members of the study team. Data entered by the participant/parent via the study web portal will be anonymised using the unique participant study identifier. All study data will be stored on University of Dundee servers with disaster recovery processes.

The DNA extraction analysis data will be uploaded via an encrypted secure file sharing process or direct secure NHS email from Professor Colin Palmer, Chair of Pharmacogenomics at the Medical Research Institute, and University of Dundee/ or delegate to the trial co-ordinator /study team.

8 TRIAL OR STUDY MANAGEMENT AND OVERSIGHT ARRANGEMENTS

The Trial Management Group will conduct trial oversight. It will be responsible for strategic decision making and monitoring ethical and participant safety issues, together with monitoring recruitment and data quality matters.

8.1 TRIAL/STUDY MANAGEMENT GROUP

The PACT trial will be managed by Tayside Clinical Trials Unit (TCTU). The Trial Management Group (TMG) will comprise of the CI, study co-ordinator, relevant PIs and co-applicants, trial statistician, TCTU Assistant Director, a representative of TCTU's data management staff, TCTU senior trial manager and study research fellow. Other members of the TMG group may be added as required by the consortium or the Trials Unit. The TMG will meet at least quarterly either face-to-face or via teleconference and more often if needed, especially in the early phases of the trial.

8.2 TRIAL/STUDY MANAGEMENT

Each clinical site will have an associated research nurse/research associate allocated to the study or delegate who will be able to discuss the project with the participant/parent and see participants face-to-face or over the telephone if required. These individuals will be accountable to the local PI or directly to the CI. The research nurse/associate will check paper or electronic data capture for completeness, plausibility and consistency. Any queries will be resolved by discussion with the CI, or delegated member of the study team, the participant and/or parent/guardian.

8.3 INSPECTION OF RECORDS

The CI, PIs and all institutions involved in the study will permit study related monitoring, audits and REC review. The CI and PIs agree to allow the Sponsor or, representatives of the Sponsor, direct access to all study records and source documentation.

9 GOOD CLINICAL PRACTICE

9.1 ETHICAL CONDUCT OF THE STUDY

The study will be conducted in accordance with the principles of good clinical practice (GCP).

In addition to Sponsorship approval, a favorable ethical opinion will be obtained from the appropriate REC and appropriate NHS R&D approval(s) will be obtained prior to commencement of the study.

Any proposed amendments to the research study will be discussed with the Sponsor. The Sponsor will determine whether the amendment is substantial or not and determine which regulatory authorities require to review and approve the protocol amendment. The CI is responsible for ensuring the amendments are implemented locally and important modifications are communicated to relevant parties when applicable.

9.1.1 CONFIDENTIALITY

Each participant will be allocated a unique study identifier via the HIC Recruitment Tracker. Thereafter, this unique identifier will be used to identify the participant. Laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain participant confidentiality. All records will be kept in a secure storage area with limited access to study staff only. All electronically data will be held under strict data security and disaster recovery plans as per HIC's SOPs and policies. All services provided by HIC are delivered within an ISO 270001 certified secure environment to ensure data is managed safely and in compliance with Data Protection legislation.

Clinical information will not be released without the written permission of the participant, except as necessary for monitoring and auditing by the Sponsor or its designee. The CI and the study team will not disclose or use records or data for any purpose other than management of the study. Prior written agreement from the Sponsor or its designee will be obtained for the disclosure of any said confidential information to other parties. The participant contact details held on the Recruitment Tracker will be archived as per HIC SOP/Data policies on project completion.

9.1.2 DATA PROTECTION

The CI and study staff involved with this study will comply with the requirements of the Data Protection Act 1998 with regard to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles. The CI and study staff will also adhere, if appropriate, to the current version of the NHS Scotland Code of Practice on Protecting Participant Confidentiality and Confidentiality: NHS Code of Practice in England. Access to collated participant data will be restricted to the CI and appropriate study staff.

Computers used to collate the data will have limited access measures via user names and passwords.

Published results will not contain any personal data that could allow identification of individual participants.

9.1.3 INSURANCE AND INDEMNITY

University of Sussex is the Sponsor of this clinical trial.

Insurance – The University of Sussex has insurance in place to cover the legal liabilities in respect of this study.

Indemnity The Sponsor does not provide study participants with indemnity in relation to participation in the study but has insurance for legal liability as described above.

10 STUDY CONDUCT RESPONSIBILITIES

10.1 PROTOCOL AMENDMENTS, DEVIATIONS AND BREACHES

The CI will seek approval for any amendments to the Protocol or other study documents from the Sponsor, REC and NHS R&D Office(s). Amendments to the protocol or other study documents will not be implemented without these approvals.

In the event that a CI needs to deviate from the protocol, the nature of and reasons for the deviation will be recorded in the CRF, documented and submitted to the Sponsor. If this necessitates a subsequent protocol amendment, this will be submitted to the Sponsor for approval and then to the appropriate REC and lead NHS R&D Office for review and approval.

In the event that a serious breach of GCP is suspected, this will be reported to the Sponsor immediately using the form Notification of Serious Breaches of Good Clinical Practice or the Trial protocol.

10.2 STUDY RECORD RETENTION

To enable monitoring and/or audits from the Sponsor, the investigators agree to keep records, including the identity of all participating participants (sufficient information to link records, all original signed informed consent forms/online consent forms and study documentation and records. The records should be retained by the study site coordinators and investigator for a period of 10 years and archived.

The electronic data collected by HIC from participants will be securely transferred to the CI post statistical analysis for long term archiving as per Sponsor’s SOP/Policies.

10.3 END OF STUDY

The end of study is defined as last participant last visit (LPLV). The Sponsor, CI and/or the TMG have the right at any time to terminate the study for clinical or administrative reasons.

The end of the study will be reported to the Sponsor and REC within 90 days, or 15 days if the study is terminated prematurely. The CI will ensure that any appropriate follow up is arranged for all participants.

A summary report of the study will be provided to the Sponsor and REC within 1year of the end of the study.

11 REPORTING, PUBLICATIONS AND NOTIFICATION OF RESULTS

11.1 PROGRESS REPORTS

Regular progress reports will be produced as required by the Ethics Committee and the study’s funder.

11.2 AUTHORSHIP POLICY

Ownership of the data arising from this study resides with the study team and their respective employers. On completion of the study, the study data will be analysed and tabulated, and a clinical study report will be prepared.

11.3 PUBLICATION

A research summary will be provided to participants/parents if they wish to be made aware of this (via the ICF).

The clinical study report will be used for publication and presentation at scientific meetings. Investigators have the right to publish orally or in writing the results of the study.

Summaries of results will also be made available to Investigators for dissemination within their clinics (where appropriate and according to their discretion.)

11.4 PEER REVIEW

This trial has undergone peer review by external peer reviewers commissioned by the funder. In addition, peer review of the protocol will occur via the University of Sussex and the resulting publication by the referees of the journals to which the papers (and its protocol) will be submitted.

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