Tailored second line therapy in asthmatic children with the arginine-16 genotype

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TAILORED SECOND LINE THERAPY IN ASTHMATIC CHILDREN WITH THE ARGININE-16 GENOTYPE

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Running Head: Genotype tailored controller therapy

ABSTRACT

The arginine-16 beta-2 receptor genotype confers increased susceptibility to exacerbations in asthmatic children taking regular long acting beta-2 agonists. We therefore evaluated using montelukast as an alternative to salmeterol as tailored second line asthma controller therapy in children expressing this susceptible genotype. 62 persistent asthmatic children with the homozygous arginine-16 genotype were randomized to receive salmeterol 50ug bid or montelukast 5/10mg od as add on to inhaled fluticasone for 1 year.
School absences (the primary outcome) were reduced with montelukast arm compared to salmeterol: difference in score = 0.40 (95%CI 0.07-0.87) p=0.005. Albuterol use was also reduced with montelukast compared with salmeterol: difference in score = 0.47 (95%CI 0.16-0.79) p<0.0001. Greater improvements occurred in both symptom and quality of life scores with montelukast vs salmeterol, while there was no difference in FEV1.
Montelukast may be suitable as tailored second line controller therapy instead of salmeterol in asthmatic children expressing the susceptible arginine-16 genotype - moving towards a personalised medicine approach to management.

ABBREVIATIONS USED

ARG-16 Arginine amino acid polymorphism at position -16 of the beta2 receptor
BTS: British Thoracic Society
LABA: Long-acting β_2 –agonists
LTRA: Leukotriene receptor antagonist
ML: Montelukast (plus fluticasone - as Flovent Diskus)
SM: Salmeterol (plus fluticasone - as Advair Diskus plus placebo for montelukast)
PEFR: Peak expiratory flow rate
FEV_1: Forced expiratory volume in 1 second
FVC: Forced vital capacity
PAQLQ: Pediatric Asthma Quality of Life Questionnaire
OR: Odds ratio
CI: Confidence interval

INTRODUCTION

Asthma is a common chronic illness in children[1]. Initial treatment consists of albuterol used on demand at step 1 of British Thoracic Society (BTS) guidelines. Regular anti-inflammatory 'controller' therapy starts with regular of inhaled corticosteroids at step 2[2]. For inadequate asthma control on step 2, inhaled long-acting beta-2-agonists (LABA) such as salmeterol, or leukotriene receptor antagonists (LTRA) such as montelukast are added at step 3 as an alternative to increasing the dose of inhaled corticosteroid. Appropriate measures of asthma control include the occurrence of day-to-day asthma symptoms, 'breakthrough' asthma attacks, the need for 'reliever' treatment with short-acting beta-2 agonists, and quality-of-life.

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[3]. However, in real life, the efficacy of salmeterol at step 3 for improving asthma control in individual children appears rather variable, and many children continue to experience day-to-day symptoms and exacerbations[4, 5]. Recently, the US Food and Drug Administration have raised concerns regarding the safety of prolonged LABA exposure, particularly highlighting the increased occurrence of worse exacerbations in children compared to adults[6].

Despite the use of regular inhaled salmeterol, a proportion of children with asthma continue to experience inadequate asthma control. In our cohort (n=1182), 50% of children on regular salmeterol experienced asthma exacerbations over a 6 month period, and 18% required inhaled albuterol 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 Arginine (ARG) 16 allele on the beta-2 receptor gene (1.7 fold), in asthmatic children exposed to regular salmeterol in conjunction with inhaled corticosteroids[4, 5]. This led us to hypothesise that, contrary to the observations on the overall population of children where salmeterol is superior in efficacy to montelukast at step3[7], those children possessing susceptible Arg16 beta-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 ARG-16 polymorphism (i.e. homozygous ARG genotype (~ 15%) who would be at potentially the greatest risk. The mechanism for worse control with regular salmeterol involves a greater susceptibility to agonist induced down regulation and uncoupling of airway beta-2 receptors and associated sub-sensitivity of response in the ARG-16 genotype[8].

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 to salmeterol when used as tailored second line controller therapy as add-on to inhaled fluticasone. The rationale is to provide evidence to support the potential for a personalised medicine based on the individual genotype to improve long term outcomes and cost effectiveness.

METHODS

Participants

The BREATHE database[4, 5] was used to identify children (5-18 years) homozygous for the Arg16 polymorphism for participation in this study. We recruited 62 children currently on regular inhaled corticosteroids as preventer medication for asthma (table 1). All participants had a history of at least one of the following as a result of asthma within the previous year: school absences, course of oral steroids, out-of-hours unscheduled visits to primary/secondary care, or hospital admissions. Children with other diseases (e.g. cystic fibrosis) were excluded. After screening for inclusion and exclusion criteria, written informed consent was obtained from the participants and/or parent/guardian as relevant. The study was approved by the Tayside Research Ethics Committee and registered with ClinicalTrials.gov:NCT00655616.

Study design

This was pragmatic randomized controlled trial. Participants were randomized into one of two treatment groups at the screening visit - Flixotide (Flovent: fluticasone propionate) via Accuhaler (Diskus) dry powder inhaler device (Allen and Hanburys, Uxbridge, UK) as per current inhaled steroid dose, plus active oral montelukast 5/10mg (Merck Sharpe and Dohme, Hoddesdon, UK); or Seretide (Advair: salmeterol 50ug bid plus equivalent dose of fluticasone) via Accuhaler (Diskus) dry powder inhaler device as per current inhaled steroid dose, plus placebo for montelukast (Figure 1). A concealed web-based randomization design was used. The investigators and participants were blinded until the participants were assigned to one of the treatments. The existing inhaled steroid doses were maintained unchanged throughout the study as the fluticasone equivalent dose – either as Flixotide or Seretide Accuhaler - along with either active or placebo montelukast respectively. This pragmatic design aimed to cause minimal change to the child's existing therapy. It also allowed ease of dispensing of the standard inhalers containing the required dose of medication through primary care. As such this design could not incorporate the blinding of the Flixotide or Seretide inhalers ,which were therefore given as open label . Inhaled salbutamol (albuterol) 2puffs (200ug) via pressurized metered dose inhaler and 750ml Volumatic spacer device was used as rescue medication.

Procedures

At baseline visit, all participants underwent detailed clinical examination. Exhaled nitric oxide (Aerocrine Mino, Solna, Sweden) and pulmonary function (Micromedical, Rochester, UK) were measured at baseline. Patients were given an asthma symptom diary to record controller and reliever medication use and exacerbation symptoms. Participants returned every 3 months for diary review, medication compliance review, spirometry, exhaled nitric oxide testing and safety and efficacy assessments. The incidence of adverse events was recorded. Serious adverse events were reported according to protocol. Compliance was monitored by viewing counters that calculated the number of actuations used from the Accuhaler. The diary cards were used as a secondary compliance check.

School absence, prospectively measured as individual events over 1 year, constituted the primary outcome measure. Secondary outcome measures included asthma-related hospitalisations, requirement of courses of oral steroids, total asthma exacerbations, the use of inhaled bronchodilator as reliever, daily asthma symptoms as reported by the participants, quality of life as measured by the pediatric asthma quality of life questionnaire PAQLQ, nitric oxide levels and spirometry.

Numerical scoring systems were used to compare school absence, inhaled bronchodilator use, asthma symptoms and quality-of-life between the two groups[4, 5]. Asthma-related school absence was numerically scored (0 = none, 1 = 1-2 days, 2 = more than 2 days and up to 1 week; 3 = more than 1 week since previous visit). The total exacerbation score was defined as the number of any of the 3 events (school absence, oral steroid course, hospital admission) since the previous visit.

Inhaled bronchodilator use was numerically scored as 0= none, 1= occasional (more than once a week and less than daily use), 2= daily, 3= excessive use (more than one dose of 200 micrograms/day for symptom control). The self-reported asthma symptoms (cough, wheeze and dyspnoea at morning and night-time) were also recorded for the period since previous visit (0=no symptoms, 1=once or twice per month, 2=once or twice weekly, 3=daily symptoms). The scores ranged from 0-3 in both groups for each of the outcomes. The standardized version of the pediatric asthma quality of life questionnaire [9] was used. The quality of life score results are expressed as the mean score per item for each of the domains, with higher scores indicating better quality of life. The minimal clinically important difference is 0.5.

Statistical analysis

A priori calculations of sample size were performed. Our initial Tayside data set showed that 85% of Arg16 homozygous asthmatic children on regular salmeterol had one or more school absences over 6 months, compared to 25% in those on inhaled steroids alone (i.e. a 60% difference). A sample of 30 patients in each arm was required to show a minimal important difference of 60% in school absences over 1 year as the primary outcome for comparison between the 2 groups, to achieve at least 80% power, with alpha error of 0.05 (2 tailed). Comparisons were made by repeated measures analysis of variance for longitudinal data measurements. Outcome variables based on daily symptoms and diary records were averaged over all the days between clinic visits. Statistical analyses by intention-to-treat were performed using SPSS for Windows version16 (SPSS Inc, Chicago, Ill) and Prism (GraphPad Software Inc).

RESULTS

The baseline characteristics of the participants are described in table1 while Figure 1 describes the trial protocol. 154 children with the homozygous Arg16 genotype were initially screened for eligibility. 62 children agreed to participate in the study (Figure 2). A significant difference was observed between pre-treatment asthma-related oral steroid requirements in the previous year ($p=0.011$) between the two treatment groups which was factored into the ANCOVA model. There were no significant baseline differences in other outcomes including school absences, FEV1%, salbutamol use, symptoms and quality of life score. The montelukast group had a higher percentage of patients at step 4 than the salmeterol group (51% vs 24%), while the converse was seen at step 3 (11% vs 35%). There was no difference in inhaled corticosteroid dose which was kept constant throughout the study – the mean (SEM) doses of FP were 299ug (31) vs 261ug (33) for montelukast vs salmeterol groups respectively.

For the primary outcome of school absences, there was a significant reduction in the ML group versus the SM group :difference in scores: 0.40 (95% CI-0.07-0.87) p=0.005 (Figure 3) over the 12 month period, while there was also a significant difference in exacerbation scores in the ML compared to the SM group was: 0.39 (95%CI-0.20-0.99) p=0.049.

Salbutamol use was significantly decreased in the ML compared to the SM group: a 0.47 difference in scores: (95%CI 0.16-0.79) p<0.0001 (Figure 3). During the study period, daily use of albuterol as reliever in the participants in the SM group did not alter over time (baseline 32%, 3 months 38%, 6 months 32%, 9 months 38%, 12 months 35%). However, in the ML group, the requirement short-acting β_2-agonists decreased over the study period (baseline 36%, months 18%, 6 months 14%, 9 months 11%, 12 months 18%). There was no significant difference in FEV_1 between the two groups: mean difference 5.46% (95% CI: -1.43 - 12.35%) (Figure 3). Early morning symptoms were significantly better in the ML compared to the SM group: mean differences: morning cough 0.51(95%CI 0.09-0.920) p=0.001; morning wheeze 0.55 (95% CI 0.25-0.86) p<0.0001; morning dyspnoea 0.29 (95%CI 0.06-0.5) p=0.0009 (Figure 4). Nocturnal wheeze and dyspnoea were improved in ML compared to SM groups: difference in scores: nocturnal wheeze 0.46 (95%CI 0.15-0.77)p=0.004, nocturnal dyspnoea 0.44 (95%CI 0.16-0.73) p=0.001 (Figure 4).

Over the year, significant differences in mean and individual domains were observed for asthma quality-of-life scores (Figure 5): the overall score was improved by 0.53 (95%CI -0.864- -0.189) p=0.003 in ML vs SM groups, symptom score: -0.53(95%CI -0.92- -0.14 p<0.0001, emotional function score -0.523(95%CI -0.84- -0.20) p<0.0001, and activity limitation score: -0.55(95%CI -0.918- -0.18) p<0.0001 (Figure 5).

Mean values for exhaled nitric oxide were halved in ML with no reduction in the SM group mean baseline to end of study: 29.3 ppb to 15.3 (ML) and 29.2 ppb to 32.6 (SM) although the difference was not significantly different. There were no serious adverse events during the study period in either group.

DISCUSSION

This is the first prospective randomized controlled study in children with asthma that addresses personalized medicine based on genotype. The results of the present study show that in children expressing the arginine genotype, in comparison to salmeterol, adding montelukast to inhaled fluticasone significantly reduced school absences, improved asthma symptoms and quality-of-life, while reducing inhaled reliever use, along with no difference in FEV_1. The relative benefits of montelukast in comparison to salmeterol became evident within the first 3 months and persisted throughout the whole year.

The children who were randomized to receive montelukast had significantly more oral corticosteroids over the year previous to enrollment suggesting the presence of more severe asthma at baseline. We factored the difference in oral corticosteroid use at baseline into the ANCOVA model. One might argue that there was more potential room for improvement in the montelukast compared to the salmeterol group if the former had more severe disease. Pointedly it was evident in terms of other key disease markers that there were no significant differences at

baseline with regards to school absences, salbutamol use, symptoms and quality of life scores, all of which improved with the addition of montelukast but not salmeterol. Furthermore both groups were closely matched (montelukast vs salmeterol) in terms of the mean daily dose of fluticasone (299ug vs 261 ug), FEV1 (87.8% vs 88.7%) and FeNO (29.3 vs 29.2 ppb) – the latter in particular suggesting no difference in asthmatic inflammation.

A recent study by Lemanske et al[3] demonstrated that it is difficult to be powered on a post hoc basis to explore putative genotype differences. In addition, their asthmatic children were required to exhibit significant reversibility to inhaled beta-agonist, hence biasing towards salmeterol responders. Furthermore, less than a quarter of the children in the Lemanske study were non-Hispanic white (with 60% of Black, Latino or Hispanic origin), whereas all of our enrolled children were of Caucasian white origin-these ethnic differences are associated with differences in beta-2 receptor haplotype variation[10, 11].

Two separate prospective genotype stratified studies in adults did not demonstrate an effect of Arg16 genotype on the primary outcome of pulmonary function in patients receiving salmeterol or placebo as add-on to inhaled corticosteroids-pointedly neither study was powered to look at exacerbations[12, 13]. Moreover the study of Bleecker et al[13] was biased towards salmeterol response as the patients were required to exhibit albuterol reversibility at screening, with a mean overall reversibility of 19%. In other words those patients who are poorly responsive to β_2-agonists were already excluded. In our experience in the pediatric service clinic, FEV_1 % predicted values tend to be well preserved in children with persistent asthma and consequently there tends to be relatively little room for further bronchodilator response to beta-agonists. Indeed, the mean FEV_1 was 88% for our study in children, compared to 79% in LARGE and 82% in Bleecker both in adults[12, 13]. In this regard we found no significant difference in FEV1 over the 12 month study period ,when comparing the two randomised treatment arms. This in turn indicates that following FEV1 with montelukast has little bearing with regards to its disease modifying activity. It is possible the development of sub-sensitivity to the non-bronchodilator (e.g. mast cell mediated) actions of inhaled long-acting β_2-agonists in the Arginine-16 genotype may have resulted in worse outcomes in the salmeterol treated patients[14-16].

In our previous studies[4, 5], we had only investigated two of the SNPs in the beta-2 receptor *2*gene, namely the Arg/Gly variation at position 16 and the Gln/Glu variation at position 27. Of these, we noted that only the Arg/Gly variation interacts with LABA resulting in an increased risk of asthma exacerbations. Other haplotypes of the *ADRB2* gene have been described[11, 17]. However, an analysis of this haplotype data shows that only three of the haplotypes are found in relevant numbers in the Caucasian population and these are completely tagged by codon 16 and codon 27. It is relevant that the promoter polymorphisms are in complete linkage disequilibrium with the Arg16 variant in the Caucasian population. Hence, in our study for which all participants were of Caucasian origin, it was not relevant to pursue a stratified design involving haplotypes of the beta-2 receptor gene.

We did not evaluate the glycine homozygous genotype (about ~ 40%) because we considered that salmeterol would be equally effective in such patients. This approach of exclusively

studying the at risk Arg16 homozygous genotype has been recently reported in another trial where adding in tiotropium or salmeterol in adult asthmatics as second line controller showed non-inferiority on bronchodilator outcomes[18]. We have also shown a 1.7 fold increased exacerbation risk related to each copy of the arginine allele[4], such that heterozygotes (i.e. Arg/Gly ~45%) would also be expected to more susceptible when exposed to regular salmeterol. Larger, more definitive studies would require testing potential therapeutic options in both Arg/Gly and Gly/Gly populations of children with asthma, perhaps comparing tiotropium or montelukast as alternative second line therapy to salmeterol.

In the LARGE trial in adults[12], patients with the Arg/Arg-16 genotype experienced no benefit on airway hyper-responsiveness with salmeterol added to inhaled steroid, whereas those who were Gly/Gly-16 had a significant improvement, while changes in bronchodilator response as PEF were similar in both genotypic groups. This points to a disconnect in terms of effects of long acting beta-agonists on bronchodilator verses bronchoprotection outcomes, as has been shown in other non genotyped studies[19]. The findings of the LARGE study are supported by a retrospective meta-analysis of placebo controlled trials looking at airway hyper-responsiveness, where Arg/Arg-16 asthmatic adults fared significantly worse than Gly/Gly-16 when LABA was added to inhaled steroid[20].

In summary we have shown that asthmatic children expressing the susceptible arginine-16 genotype appear to fare better on montelukast than salmeterol when added to inhaled corticosteroid as second line controller therapy over a 12 month period. This raises the key question as to whether prior gene testing could be used to tailor appropriate second line 'controller' for patients with asthma at step 3 of asthma guidelines i.e. moving towards a personalised medicine approach to management.

CLINICAL PERSPECTIVES

- FDA have recently highlighted concerns about long term safety of long acting beta-agonist exposure especially in children
- Our study evaluated 2nd line therapy in 15% of genetically susceptible asthmatic children possessing the arginine-16 genotype
- Our study shows that patients with the arginine-16 genotype may fare better by using montelukast than salmeterol as add on therapy to inhaled corticosteroids
- This in turn provides evidence for genotype directed personalised medicine

AUTHOR CONTRIBUTIONS

KB had full access to all the data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis.
Study concept and design: BJL, SAO, CNAP, SM, DFM
Random allocation: SAO
Enrollment and assigning of participants: KB, HPD
Acquisition of data: KB, HPD
Analysis and interpretation of data: BJL, KB, HPD, CNAP, SM
Drafting of the manuscript: BJL, KB, HPD, CNAP, SM
Critical revision of manuscript for important intellectual content: BJL,KB, RT, DFM, SAO, HPD, CNAP, SM
Statistical analyses: KB, CNAP
Obtained funding: BJL, DFM, CNAP, SM
Administrative, technical or material support: KB, HPD, BJL, RT, DFM, CNAP, SM
Study supervision: BJL, DFM, CNAP, SM

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FIGURE LEGENDS

Figure1: Schematic diagram of the trial protocol. The participants were randomly assigned to continue Flixotide plus oral montelukast (ML) or Seretide plus placebo montelukast (SM), for 12 months.

Figure 2: Consort diagram showing flow of participants through the study. All patients for whom follow-up data were available were included in the analysis on an intention to treat basis.

Figure 3: Top : Change in asthma-related school absences over 1year study period between groups treated with Flixotide plus oral montelukast (ML) or Seretide plus placebo montelukast (SM).Visits were made as follows over 12 months: #0 (baseline), #1 (3months), # 2 (6months), #3 (9 months), #4 (12 months). The baseline value was obtained over the year prior to recruitment. **Middle:** Change in use of salbutamol reliever. The baseline value was obtained over the year prior to recruitment. **Bottom**: Change in FEV_1.

Figure 4: Change in the self-reported morning (**Left**) and evening (**Right**) asthma symptoms. (A) cough score, (B) wheeze score, (C) dyspnoea score. Error bars represent 95% CI's. P values are shown for the comparison between groups throughout the treatment period. The baseline value was obtained over the year prior recruitment.

Figure 5: Change in total Pediatric asthma quality of life questionnaire score (**Top**) and individual domains (**Bottom**) over 1year study period. Error bars are CI's. P values refer to the comparison between the two treatment groups throughout the treatment period.

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Table 1: Baseline Characteristics of study participants

Characteristic	Fluticasone plus salmeterol and placebo montelukast (n=34)	Fluticasone plus montelukast (n=28)	Difference (95% CI; p values) ¶¶¶
Age at randomization- years¶	11.79±3.9	10.50±3.3	
Male sex-%	56	71	
Body mass index¶†	19.35±4.3	18.04±3.7	
Use of inhaled salbutamol for asthma ≥twice/week-%	91	96	0.001(-0.36-0.36; 0.99)
Oral corticosteroids over previous year-%	20	43	-0.73 (-1.29- -0.17; **0.01*)**
Daily inhaled corticosteroids-%	100	100	
Asthma related school absences over previous year-%	92	96	0.06 (-0.38-0.51;0.77)
Asthma related hospital admissions in previous year-%	15	22	-0.04 (-0.38- 0.29; 0.79)
Pediatric asthma quality of life scores¶¶			
Symptom scores¶¶	5.67 (0.22)	5.52 (0.19)	0.15(-0.45-0.75;0.62)
Emotional function scores¶¶	6.09 (0.19)	5.91 (0.17)	0.19(-0.34-0.72;0.48)
Activity limitation scores¶¶	5.72 (0.18)	5.71 (0.16)	0.004 (-0.50-0.51; 0.99)
Asthma symptoms-% ¤			
Night wheeze%	47	71	-0.45 (-0.97-0.07; 0.09)
Night cough%	68	86	-0.51 (-1.05-0.04; 0.07)
Night dyspnoea%	38	61	-0.21 (-0.72-0.30; 0.41)
Morning wheeze%	38	43	0.07 (-0.39-0.54; 0.75)
Morning cough%	59	68	-0.06 (-0.68-0.56; 0.84)
Morning dyspnoea%	26	39	0.05 (-0.41-0.52; 0.81)
Modified British Thoracic Society (BTS) step of asthma treatment-% ††	2= 41; 3= 35; 4= 24	2= 39; 3= 11; 4= 50	
Pulmonary function¶¶			
Number of participants with measurement	34	28	
PEFR-% of predicted value	73.85(5.8)	70.86(4.9)	
FEV_1-% of predicted value	88.74(4.1)	87.8(2.8)	

FVC-% of predicted value	89.38(4.4)	88.25(2.7)	

PEFR: peak expiratory flow rate, FEV_1: forced expiratory volume in 1 second, FVC : forced vital capacity.

¶Means ± SD.

¶¶Means (SE of mean)

¶¶¶Differences between the respective pre-treatment values for the treatment groups, are presented as mean fold changes (95% CI; p values)

†The body-mass index is the weight in kilograms divided by the square of the height in meters.

¤These symptoms were self reported. 0=no symptoms, 1=once or twice per month, 2=once or twice weekly, 3=daily symptoms

††Step2= Regular inhaled steroids+ inhaled β2 agonists as and when required; Step 3= Step2+ inhaled long acting β2 agonists; Step 4= Step 3+ montelukast

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Figure 1: Schematic diagram of the trial protocol

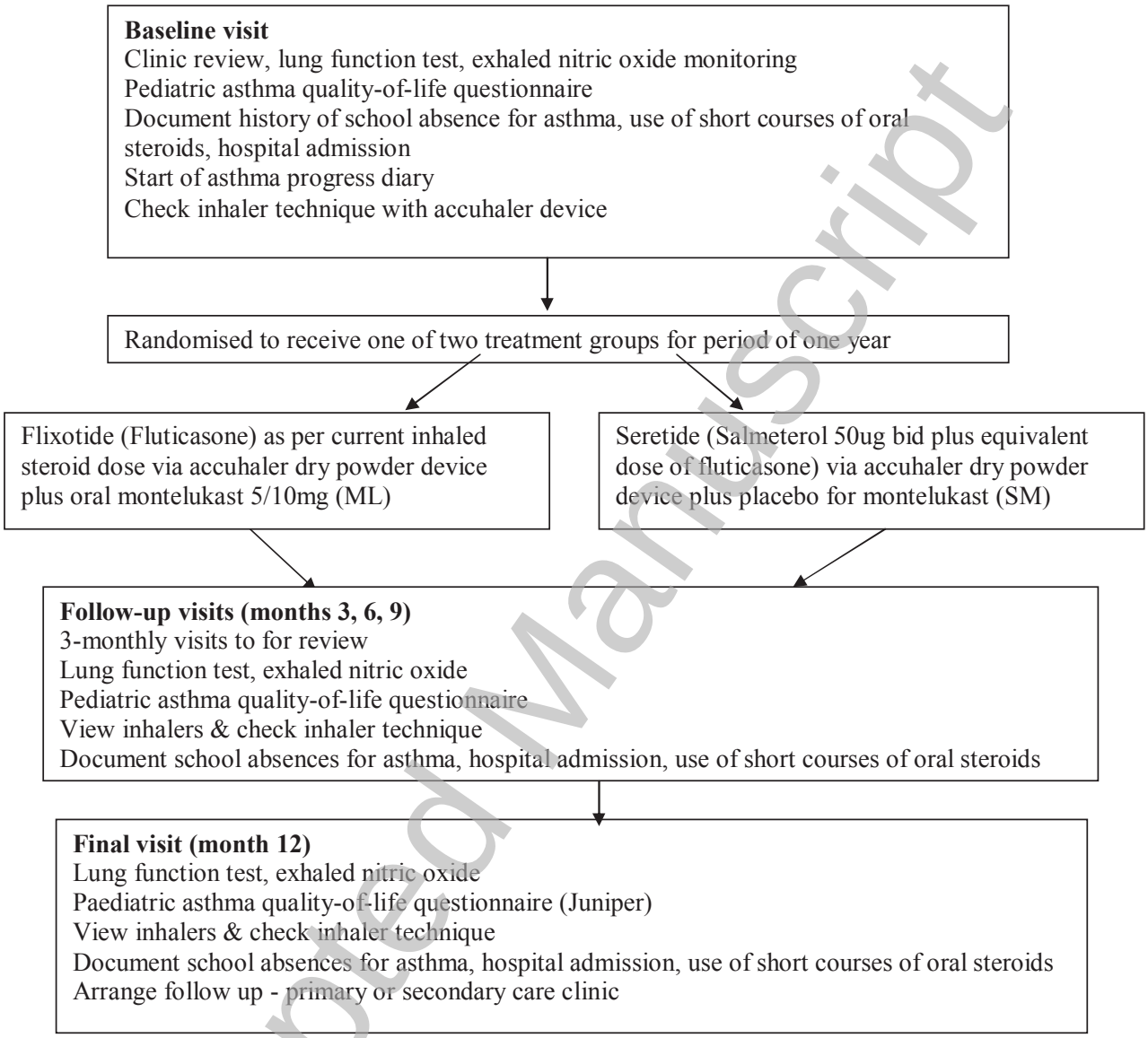

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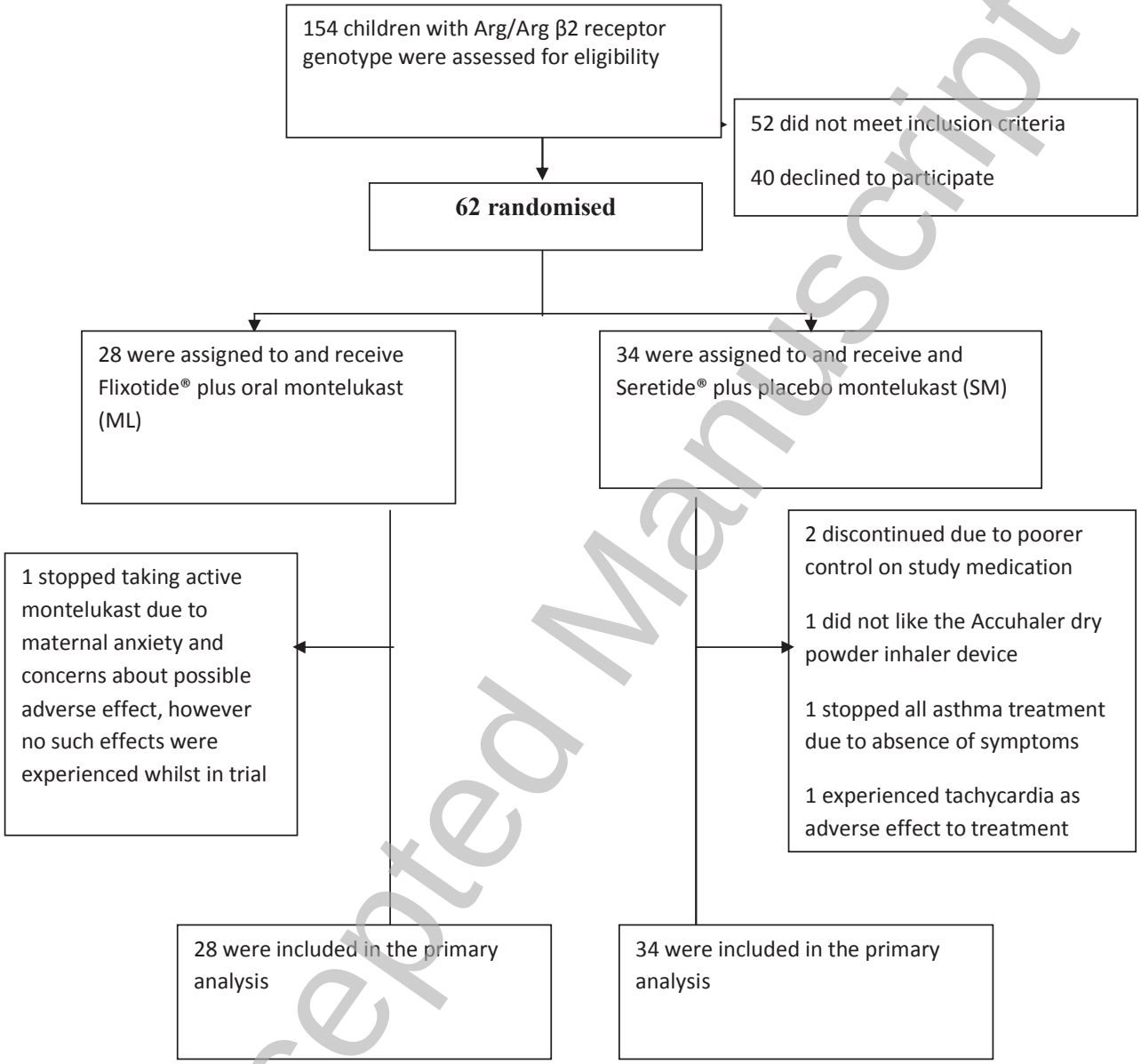
Figure 2: Consort diagram showing flow of participants through the study



All patients for whom follow-up data were available were included in the analysis on an intention to treat basis

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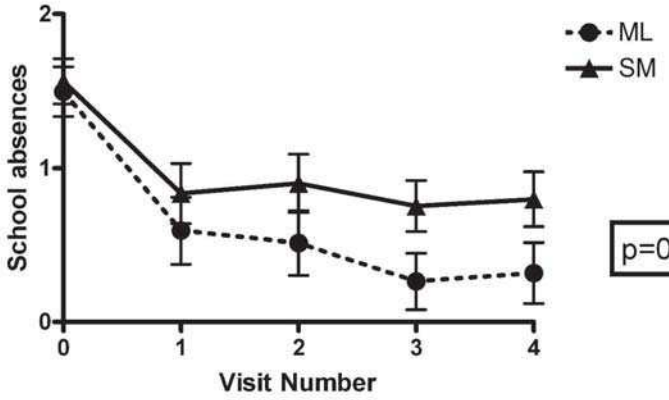

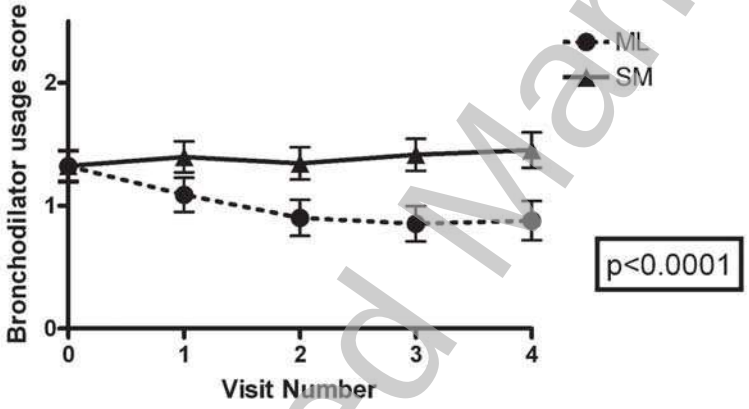

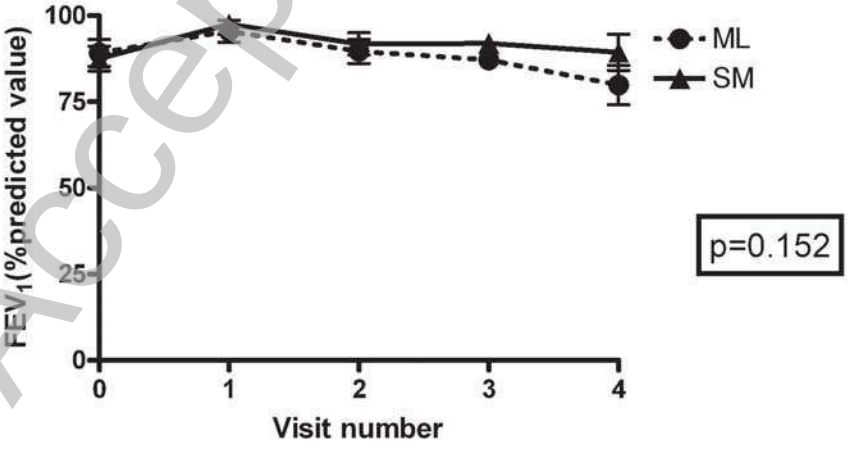

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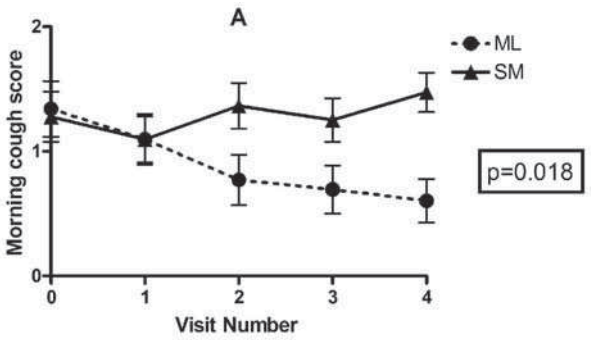

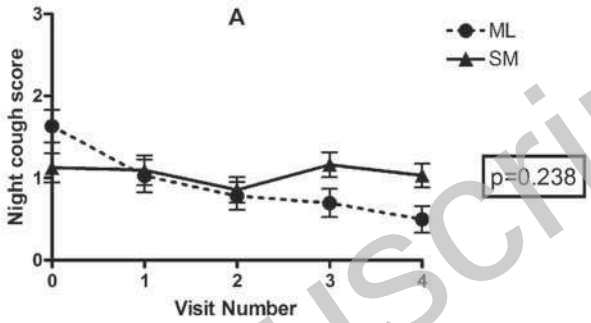

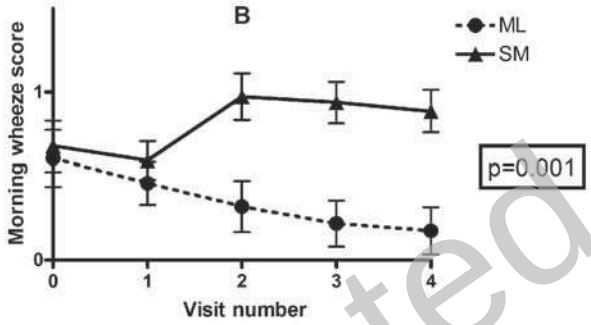

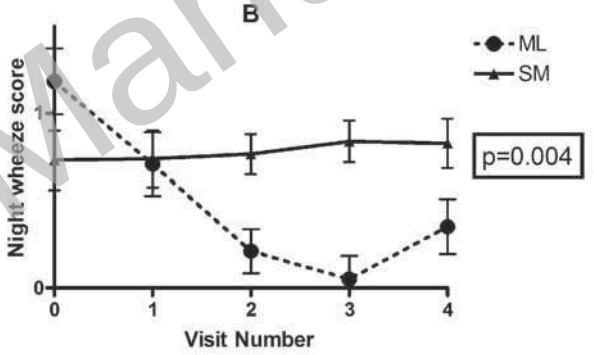

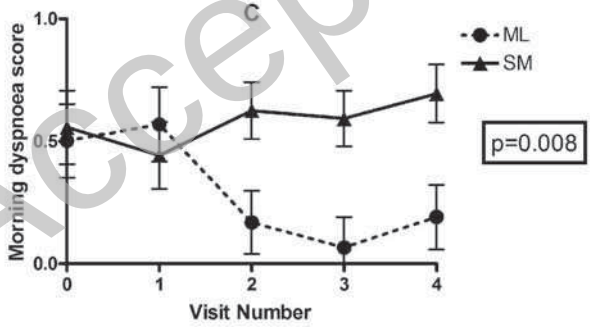

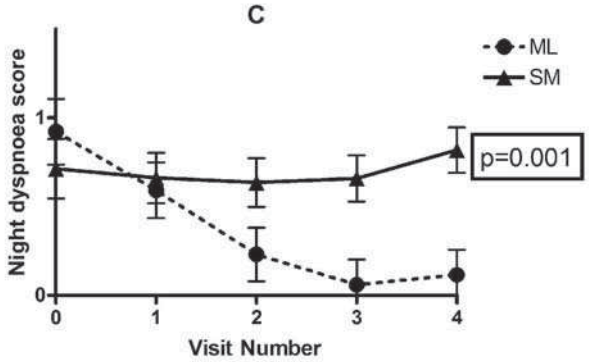

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