Tailored second-line therapy in asthmatic children with the Arg\textsuperscript{16} genotype

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Abstract

The Arg\textsuperscript{16} \(\beta_2\) receptor genotype confers increased susceptibility to exacerbations in asthmatic children taking regular LABA (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. A total of 62 persistent asthmatic children with the homozygous Arg\textsuperscript{16} genotype were randomized to receive salmeterol (50 \(\mu\)g, b.i.d.) or montelukast (5 or 10 mg, once daily) as an add-on to inhaled fluticasone for 1 year. School absences (the primary outcome) were reduced with montelukast compared with salmeterol \{difference in score = –0.40 [95% CI (confidence interval), –0.22 to –0.58]; \(P=0.005\}\). Salbutamol use was also reduced with montelukast compared with salmeterol [difference in score = –0.47 (95% CI, –0.16 to –0.79); \(P<0.0001\)]. Greater improvements occurred in both symptom and quality of life scores with montelukast against salmeterol, whereas there was no difference in FEV\textsubscript{1} (forced expiratory volume in 1 s). In conclusion, montelukast may be suitable as tailored second-line controller therapy instead of salmeterol in asthmatic children expressing the susceptible Arg\textsuperscript{16} genotype, a move towards a personalized medicine approach to management.

Key words: asthma, \(\beta_2\) receptor, children, genotype, montelukast, salmeterol

INTRODUCTION

Asthma is a common chronic illness in children [1]. Initial treatment consists of salbutamol used on demand at step 1 of BTS (British Thoracic Society) guidelines. Regular anti-inflammatory ‘controller’ therapy starts with regular corticosteroids at step 2 [2]. For inadequate asthma control on step 2, inhaled LABA (long-acting \(\beta_2\) agonists) such as salmeterol, or LTRA (leukotriene receptor antagonists) 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 U.S. FDA (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 with adults [6].

Despite regular inhaled salmeterol, a proportion of children with asthma continued 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 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 Arg\textsuperscript{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 hypothesize that, contrary to the observations on the overall population of children where salmeterol is...
superior in efficacy to montelukast at step 3 [7], those children possessing susceptible Arg16 β2 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%) 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 β2 receptors and associated sub-sensitivity of response in the Arg16 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 with 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 personalized medicine based on the individual genotype to improve long-term outcomes and cost effectiveness.

### MATERIALS AND 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 medication for preventing 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 a pragmatic randomized controlled trial. Participants were randomized into one of the two treatment groups at the screening visit: flixotide (Flovent: fluticasone propionate) via

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### Table 1 Baseline characteristics of study participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Fluticasone plus salmeterol and placebo montelukast (n = 34)</th>
<th>Fluticasone plus montelukast (n = 28)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at randomization (years)</td>
<td>11.79 ± 3.9</td>
<td>10.50 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>56</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.35 ± 4.3</td>
<td>18.04 ± 3.7</td>
<td></td>
</tr>
<tr>
<td>Use of inhaled salbutamol for asthma ≥twice/week (%)</td>
<td>91</td>
<td>96</td>
<td>0.001 (–0.36 to 0.36; 0.99)</td>
</tr>
<tr>
<td>Oral corticosteroids over previous year (%)</td>
<td>20</td>
<td>43</td>
<td>–0.73 (–1.29 to 0.01)</td>
</tr>
<tr>
<td>Daily inhaled corticosteroids (%)</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Asthma related school absences over previous year (%)</td>
<td>92</td>
<td>96</td>
<td>0.06 (–0.38 to 0.51; 0.77)</td>
</tr>
<tr>
<td>Asthma related hospital admissions in previous year (%)</td>
<td>15</td>
<td>22</td>
<td>–0.04 (–0.38 to 0.29; 0.79)</td>
</tr>
<tr>
<td>Paediatric asthma quality of life scores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptom scores</td>
<td>5.67 (0.22)</td>
<td>5.52 (0.19)</td>
<td>0.15 (–0.45 to 0.75; 0.62)</td>
</tr>
<tr>
<td>Emotional function scores</td>
<td>6.09 (0.19)</td>
<td>5.91 (0.17)</td>
<td>0.19 (–0.34 to 0.72; 0.48)</td>
</tr>
<tr>
<td>Activity limitation scores</td>
<td>5.72 (0.18)</td>
<td>5.71 (0.16)</td>
<td>0.004 (–0.50 to 0.51; 0.99)</td>
</tr>
<tr>
<td>Asthma symptoms (%)*</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Night wheeze</td>
<td>47</td>
<td>71</td>
<td>–0.45 (–0.97 to 0.07; 0.09)</td>
</tr>
<tr>
<td>Night cough</td>
<td>68</td>
<td>86</td>
<td>–0.51 (–1.05 to 0.04; 0.07)</td>
</tr>
<tr>
<td>Night dyspnoea</td>
<td>38</td>
<td>61</td>
<td>–0.21 (–0.72 to 0.30; 0.41)</td>
</tr>
<tr>
<td>Morning wheeze</td>
<td>38</td>
<td>43</td>
<td>0.07 (–0.39 to 0.54; 0.75)</td>
</tr>
<tr>
<td>Morning cough</td>
<td>59</td>
<td>68</td>
<td>–0.06 (–0.68 to 0.56; 0.84)</td>
</tr>
<tr>
<td>Morning dyspnoea</td>
<td>26</td>
<td>39</td>
<td>0.05 (–0.41 to 0.52; 0.81)</td>
</tr>
<tr>
<td>Modified BTS step of asthma treatment (%)†</td>
<td>2 = 41; 3 = 35; 4 = 24</td>
<td>2 = 39; 3 = 11; 4 = 50</td>
<td></td>
</tr>
<tr>
<td>Pulmonary function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participants with measurement (n)</td>
<td>34</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>PEFR (% of predicted value)</td>
<td>73.85 (5.8)</td>
<td>70.86 (4.9)</td>
<td></td>
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<tr>
<td>FEV1 (% of predicted value)</td>
<td>88.74 (4.1)</td>
<td>87.80 (2.8)</td>
<td></td>
</tr>
<tr>
<td>FVC (% of predicted value)</td>
<td>89.38 (4.4)</td>
<td>88.25 (2.7)</td>
<td></td>
</tr>
</tbody>
</table>

*These symptoms were self-reported (0 = no symptoms; 1 = once or twice per month; 2 = once or twice weekly; 3 = daily symptoms).
†Step 2 = regular inhaled steroids + inhaled β2 agonists as and when required; step 3 = step 2 + inhaled long-acting β2 agonists; step 4 = step 3 + montelukast.
Genotype-tailored controller therapy

The participants were randomly assigned to continue flixotide plus oral montelukast or seretide plus placebo montelukast, for 12 months. An Accuhaler (Diskus) dry powder inhaler device (Allen and Hanburys) as per current inhaled steroid dose, plus active oral montelukast (5 or 10 mg; Merck Sharpe & Dohme); or seretide (Advair: salmeterol 50 μg, b.i.d. plus an equivalent dose of fluticasone) via an 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 was 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 Accuhaler – along with either active or placebo montelukast respectively. This pragmatic design was 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.

Procedures
At baseline visit, all participants underwent detailed clinical examination. Exhaled NO (nitric oxide) (Aerocrine Mino) and pulmonary function (Micromedical) were measured at baseline. Patients were given an asthma symptoms diary to record controller and reliever medication use and exacerbation symptoms. Participants returned every 3 months for diary review, medication compliance review, spirometry, exhaled NO 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 hospitalizations, 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 PAQLQ (Paediatric Asthma Quality of Life Questionnaire), NO 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 three 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 μg/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 and 3 = daily symptoms). The scores ranged from 0 to 3 in both groups for each of the outcomes. The standardized
version of the PAQLQ [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 dataset showed that 85% of Arg<sup>16</sup> homozygous asthmatic children on regular salmeterol had one or more school absences over 6 months, compared with 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 two groups, to achieve at least 80% power, with α error of 0.05 (two-tailed). Comparisons were made by repeated measures ANOVA 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 and Prism (GraphPad Software).

RESULTS

The baseline characteristics of the participants are described in Table 1, whereas Figure 1 describes the trial protocol. In total, 154 children with the homozygous Arg<sup>16</sup> genotype were initially screened for eligibility. A total of 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 (analysis of co-variance) model. There were no significant baseline differences in other outcomes including school absences, FEV<sub>1</sub>% [percentage FEV<sub>1</sub> (forced expiratory volume in 1 s)], 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% against 24%), whereas the converse was seen at step 3 (11% against 35%). There was no difference in inhaled corticosteroid dose that was kept constant throughout the study – the means ± S.E.M. doses of FP were 299 μg (31) against 261 μg (33) for montelukast against salmeterol groups, respectively.

For the primary outcome of school absences, there was a significant reduction in the montelukast group compared with the
salmeterol group [difference in scores = 0.40 [95% CI (confidence interval), −0.22 to −0.58; P = 0.005]) (Figure 3) over the 12-month period, whereas there was also a significant difference in exacerbation scores in the montelukast compared with the salmeterol group was −0.39 (95% CI, −0.15 to −0.64; P = 0.049).

Salbutamol use was significantly decreased in the montelukast compared with the salmeterol group [a −0.47 difference in scores (95% CI, −0.16 to −0.79; P < 0.0001)] (Figure 3). During the study period, daily use of salbutamol as reliever in the participants in the salmeterol group did not alter over time (baseline, 32%; 3 months, 38%; 6 months, 32%; 9 months, 38%; 12 months, 35%). However, in the montelukast group, the requirement for short-acting β₂-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 FEV₁ between the two groups [mean difference = 5.46% (95% CI, −1.43 to 12.35%)] (Figure 3).

Early morning symptoms were significantly better in the montelukast compared with the salmeterol group [mean differences: morning cough = 0.51 (95% CI, 0.09 to 0.92; P = 0.001); morning wheeze = 0.55 (95% CI, 0.25 to 0.86; P < 0.0001); morning dyspnoea = 0.29 (95% CI, 0.06 to 0.5; P = 0.0009)] (Figure 4). Nocturnal wheeze and dyspnoea were improved in montelukast compared with salmeterol groups [difference in scores: nocturnal wheeze = 0.46 (95% CI, 0.15 to 0.77; P = 0.004); nocturnal dyspnoea = 0.44 (95% CI, 0.16 to 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, −0.19 to −0.86; P = 0.003) in montelukast against salmeterol groups; symptom score: −0.53 (95% CI, −0.14 to −0.92; P < 0.0001), emotional function score −0.52 (95% CI, −0.20 to −0.84; P < 0.0001) and activity limitation score: −0.55 (95% CI, −0.18 to −0.92; P < 0.0001)] (Figure 5).

Mean values for exhaled NO were halved with montelukast with no reduction in the salmeterol group mean baseline to the end of study: 29.3–15.3 p.p.b. (montelukast) and 29.2–32.6 p.p.b. (salmeterol), although the difference was not significant. There were no serious adverse events during the study period in either group.

**DISCUSSION**

The present study 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 with 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 FEV₁. The relative benefits of montelukast in comparison with 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 enrolment 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 room for potential improvement in the montelukast compared with 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 against salmeterol) in terms of the mean daily dose of fluticasone (299 μg against 261 μg), FEV₁ (87.8% against 88.7%) and FENO (fraction of exhaled NO) (29.3 p.p.b. (montelukast), although the difference was not significant. There were no serious adverse events during the study period in either group.
against 29.2 p.p.b.) – 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 β-agonist, hence biasing towards salmeterol responders. Furthermore, less than 25% 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 β2 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 salbutamol reversibility at screening, with a mean overall reversibility of 19%. In other words, those patients who are poorly responsive to β2-agonists were already excluded. In our experience in the paediatric service clinic, FEV1% 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 β-agonists. Indeed, the mean FEV1 was 88% for our study in children, compared with 79% in LARGE and 82% in Bleecker et al., 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 randomized 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 LABA in the Arg16 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 (single nucleotide polymorphisms) in the ADRB2 (β2 receptor) gene, namely the Arg/Gly variation at position 16 and the Gln/Glu variation at position 27. Of these, we observed 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 at the risk of Arg$^{16}$ 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 be 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 (peak expiratory flow) were similar in both genotypic groups. This indicates a disconnect in terms of the effects of LABA on bronchodilator compared with 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 Arg$^{16}$ 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 an appropriate second-line ‘controller’ for patients with asthma at step 3 of asthma guidelines i.e. moving towards a personalized medicine approach to management.

CLINICAL PERSPECTIVES

- The FDA has recently highlighted concerns about long-term safety of LABA exposure, especially in children.
- Our study evaluated second-line therapy in 15% of genetically susceptible asthmatic children possessing the Arg$^{16}$ genotype.
- We show that patients with the Arg$^{16}$ genotype may fare better by using montelukast than salmeterol as add-on therapy to inhaled corticosteroids. This in turn provides evidence for genotype directed personalized medicine.

AUTHOR CONTRIBUTION

The study concept and design was done by Brian Lipworth, Simon Ogston, Colin Palmer, Somnath Mukhopadhyay and Donald Macgregor. Simon A Ogston did the random allocation. Enrolment
and assigning of participants and acquisition of data was done by Kaninika Basu and Helen Donald. Analysis and interpretation of data and drafting of the paper was done by Brian Lipworth, Kaninika Basu, Helen Donald, Colin Palmer and Somnath Mukhopadhyay. Critical revision of the paper for important intellectual content was done by Brian Lipworth, Kaninika Basu, Roger Pavendale, Donald Macgregor, Simon Ogston, Helen Donald, Colin Palmer and Somnath Mukhopadhyay. Statistical analyses were done by Kaninika Basu and Colin Palmer. Brian Lipworth, Donald Macgregor, Colin Palmer and Somnath Mukhopadhyay supervised and obtained funding for the study. Administrative, technical or material support was given by Kaninika Basu, Helen Donald, Brian Lipworth, Roger Pavendale, Donald Macgregor, Colin Palmer and Somnath Mukhopadhyay. Kaninika Basu 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.

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