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Primary prevention of asthma in high-risk children using HDM SLIT; assessment at age 6 years

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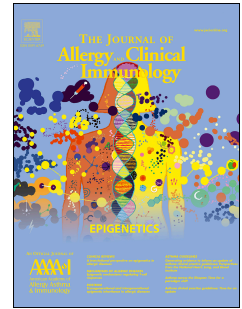
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Primary prevention of asthma in high-risk children using HDM SLIT; assessment at age 6 years

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Conflict of Interest:

G. Roberts has received research support from the National Institute for Health Research (NIHR), and National Institute of Health (NIH); he has received lecture fees from ALK-Abello and is a member of the ALK-Abello advisory board, and has a patent held by the university. Dr. Arshad reports grants from NIHR, UK; grants from National Institute of Health, USA, non-financial support and other from ALK-Abello, Denmark. In addition, Dr. Arshad has a patent for use of house dust mite allergen immunotherapy for primary prevention of asthma and atopy pending. Dr. Vijayanand reports grants from the NIH during the conduct of the study.

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Capsule summary:

Treatment of high-risk infants with HDM SLIT during early life resulted in a trend for reduction in asthma rates mid-childhood. This may be a viable preventative therapy for childhood asthma.

Key words:

Asthma

Immunotherapy

Prevention

Childhood

Randomized controlled trial

Atopy

Abbreviations

SLIT: sub-lingual immunotherapy

HDM: House dust mite

51 To the Editor,

52 Asthma has increased in recent decades worldwide with prevalence up to 15%, in
53 the US, the direct cost of uncontrolled asthma is estimated at over \$300 billion during
54 next 20 years (1). Allergic sensitization in early life, particularly multiple sensitization,
55 carries the greatest risk for later asthma (2) and therefore primary prevention must
56 target infancy, prior to sensitization occurring. Evidence has emerged that exposure
57 to a high dose of allergen early in life can drive the developing immune system
58 towards a state of tolerance (3).

59 One such strong immune stimulus is house dust mite (HDM), a potent, prevalent
60 allergen. The Mite Allergy Prevention Study (MAPS) was the first trial investigating
61 HDM sub-lingual immunotherapy (SLIT) for primary prevention of atopy in non-
62 sensitized infants (4, 5). In this proof-of-concept, double-blind, placebo-controlled
63 study, one year of HDM SLIT led to a significant reduction in any sensitization
64 compared to placebo. The treatment was safe and acceptable to families. We report
65 the results of assessment at age 6.5 years, five years after cessation of
66 immunotherapy.

67 The study was carried out as described in the Supplementary Material. Briefly, 111
68 infants, aged 5 months and at high risk of atopy (≥ 2 first-degree atopic relatives) but
69 with negative skin-prick tests (SPT) were recruited and randomized to either oral
70 HDM or saline placebo twice daily for 12 months. At 6 years participants underwent
71 assessment for asthma, a *priori* primary outcome. Validated questionnaires were
72 used for wheeze, as well as rhinitis, food allergy and eczema. They underwent SPT
73 to nine allergens and an extensive respiratory assessment including spirometry with
74 reversibility, exhaled nitric oxide and methacholine bronchial hyper-responsiveness.

This information was used to diagnose definite or likely asthma by an adjudication committee of three blinded, experienced, independent clinicians (see Supplementary Material for definitions). An observational birth cohort “Immune Tolerance in Early Childhood” (ITEC) of high risk infants was also recruited at the same time as MAPS. These infants were similar to the MAPS participants at baseline, and assessed identically at age 6. An intention to treat analysis was undertaken using Stata software, version 14 (StataCorp, College Station, USA) and GraphPad Prism v7 (San Diego, USA).

Of the 111 infants originally randomized into study (Fig S2), 41 participants in the SLIT (71.9%) and 44 participants in the placebo group (81.5%) completed the assessment at age six. The groups were well-balanced, although the placebo group had a longer duration of breastfeeding and less pet exposure at home compared to the SLIT group at baseline (Table S1). The ITEC participants were similar to the MAPS placebo group except for a shorter breastfeeding duration and younger age at baseline (Table S2).

A trend for lower rates of asthma (primary outcome) was noted in the SLIT group. For definite asthma, there was 1 case (2.9%) in the SLIT and 5 (13.5%) in the placebo groups (10.6% difference, 95% CI 23.0%, -1.8%, $p = 0.11$). For likely asthma, 4 (10.8%) participants in the SLIT and 8 (20.0%) in the placebo were affected ($p = 0.27$) (Figure 1A). There was no difference between the groups in lung function, FeNO and BHR (Supplementary Material), or other atopic outcomes (Table S4).

In a secondary analysis, incorporating ITEC cohort with MAPS placebo group, to increase power, significantly lower rates of definite asthma were seen in the SLIT

group (1 case [2·9%]) compared to 26 [16·8%]), [13·8% difference, 95% CI 22·0%, 5·7%, $p = 0·04$] (figure 1B), and a trend for less “likely asthma” was noted (13·6% difference, 95% CI 26·2%, -2·3 %, $p = 0·07$). Removing from analysis the 12 ITEC participants sensitized at baseline did not alter the results (Table S6).

For the secondary outcome of any allergic sensitization a trend was maintained for reduced sensitization in the SLIT group compared to placebo ($p = 0·07$) (Figure 2A), in a time-to-event analysis. At age six, 15 (27·8%) children were sensitized in the SLIT compared to 24 (45·4%) in the placebo group, (18·2% difference [95% CI, 35·1% to -1·8%, $p = 0·06$]). No difference was seen in rates of sensitization to HDM alone (Figure 2B, Table S5).

We have mirrored the early food allergen exposure inducing tolerance approach with HDM for respiratory allergies. HDM allergen was selected as it is a potent immune reactor modulating the developing immune system through allergenic, endotoxin and enzymatic effects with a bystander effect on other allergens (6)..

A recent meta-analysis on the use of AIT as primary immunoprophylaxis was inconclusive (7). This was ascribed to the limited body of evidence of the short term-effect of AIT on prevention, with no published RCTs investigating the long-term preventive effects of AIT. Previous studies have failed to demonstrate a role for the use of immunotherapy in the prevention of asthma and sensitization in young children (8, 9). However, the populations studied were older, and already sensitized to at least one allergen.

Our study was a small, single-centre proof-of-concept study and therefore has a number of limitations. Sample size was small, and the study was only powered to assess correct ordering of sensitization. It may also have been more effective to use

a larger allergen dose and longer duration of treatment, although the allergen dose used has been shown to induce immunological response . Nonetheless, we established safety, feasibility and preliminary proof of efficacy of SLIT in this young population.

To increase power of analysis a comparison was made between SLIT group and the placebo group combined with ITEC participants as additional controls. Both MAPS and ITEC participants were recruited in parallel from the same population and the two cohorts were highly comparable, except breast feeding was more frequent in the MAPS placebo group than ITEC cohort. As SLIT participants were also breast fed less than the MAPS placebo group, combining the MAPS placebo and ITEC cohort equalized breastfeeding rates.

In summary, this remains the only double-blind, placebo-controlled study investigating primary prevention of asthma and atopy using allergen immunotherapy, demonstrating that early life administration of HDM SLIT may reduce childhood asthma. The results seen when the larger ITEC study is incorporated into the analysis gives credence to this conclusion. Further, adequately powered studies are now required to assess the efficacy of this approach.

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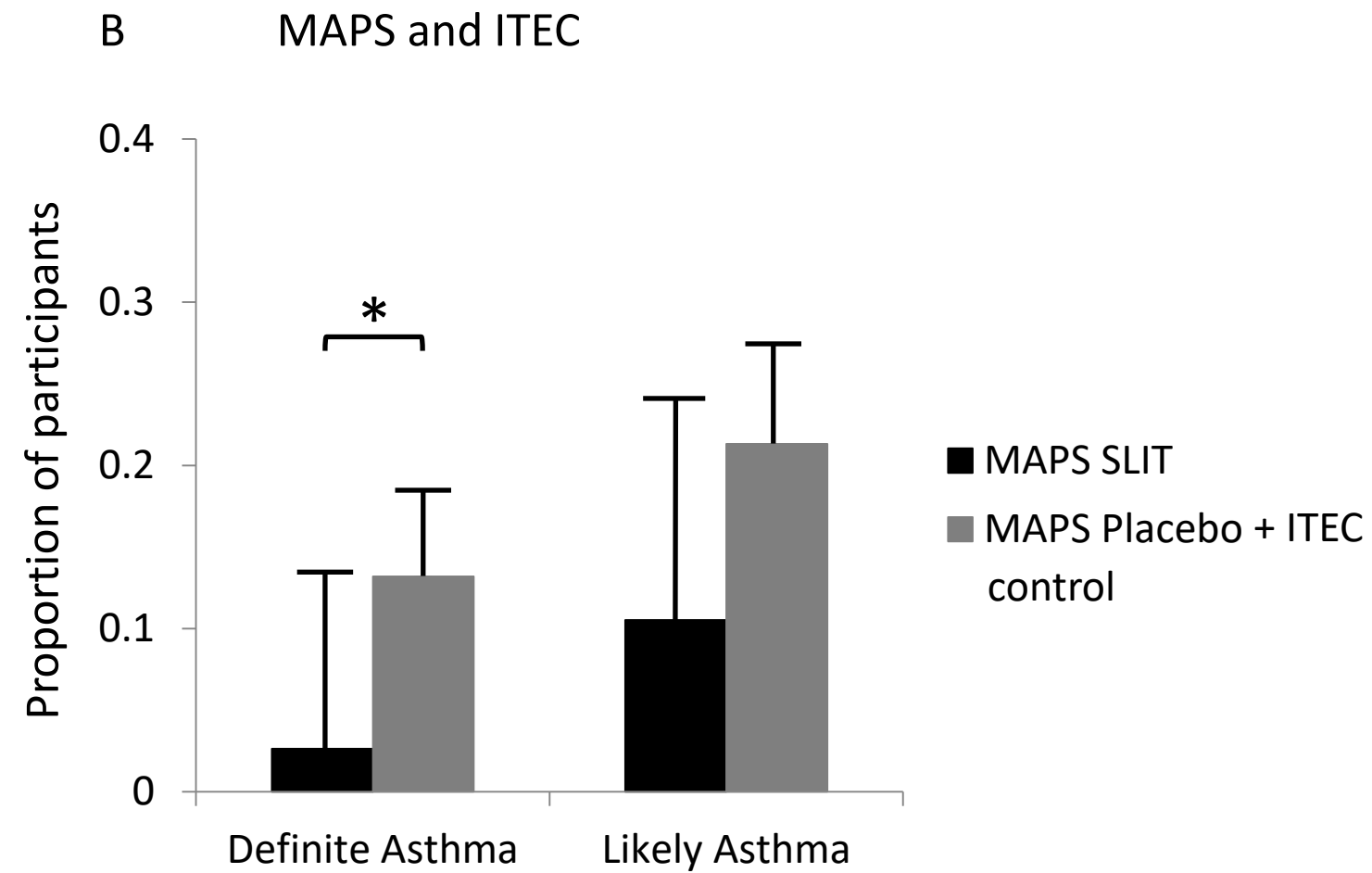
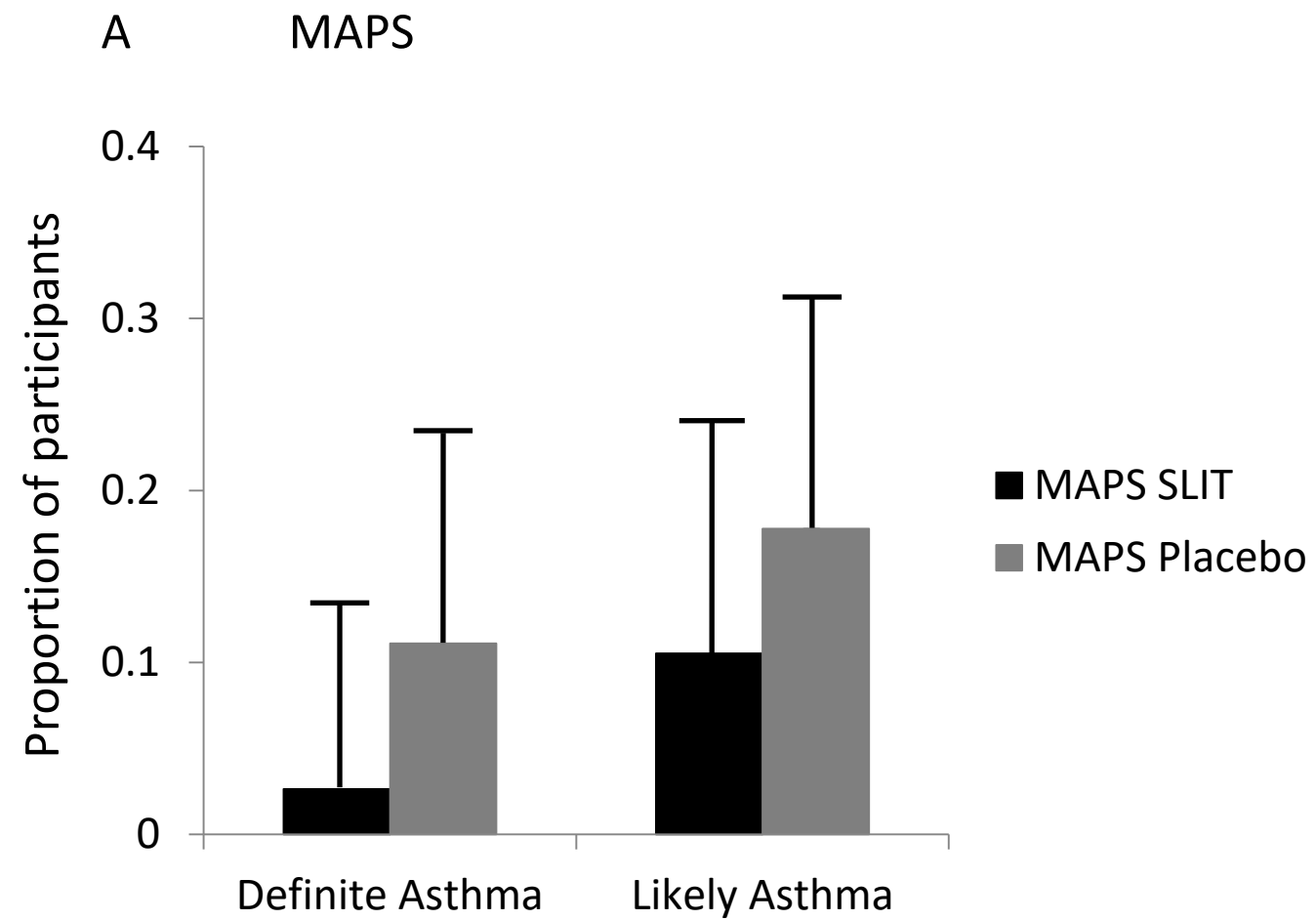
Figure Legend

Figure 1: Comparison of definite asthma and likely asthma (definite + probable) diagnosis between: A MAPS SLIT and MAPS placebo groups; B MAPS SLIT and MAPS placebo + ITEC control groups. Bars represent proportion of participants within group, with 95% CI bars. *= p <0.05.

Figure 2: Kaplan-Meyer plot comparing SLIT and placebo participants' cumulative sensitization to any common allergen (A) or HDM alone (B) over the entire study follow-up period up.

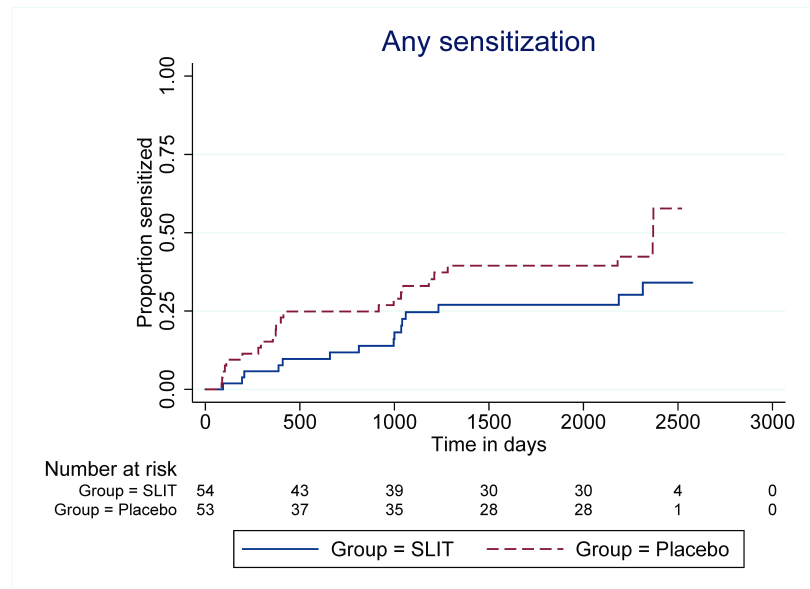
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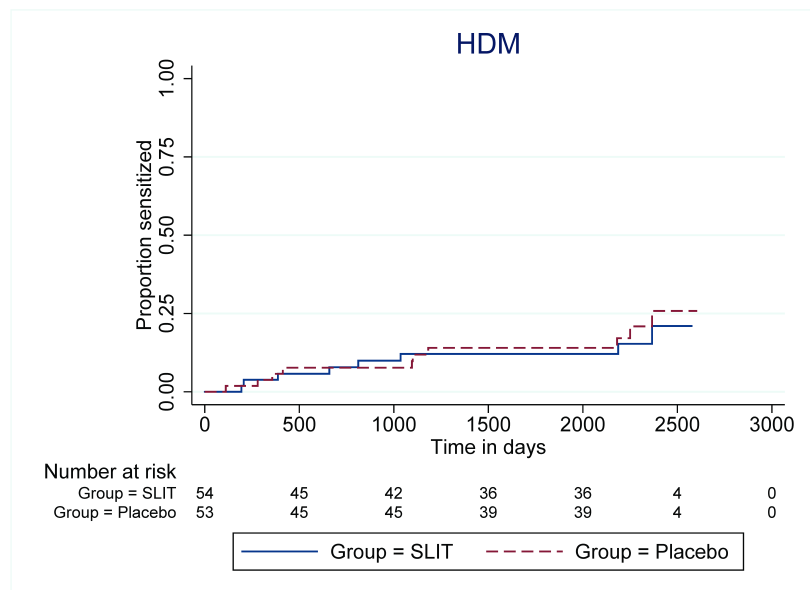


A

Log rank
test : Chi
squared
test = 3.23
p = 0.07

**B**

Log rank
test : Chi
squared
test = 0.18
p = 0.68



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Online supplementary file

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METHODS

Study design

This was a prospective, randomized, double-blind, placebo-controlled phase IIa study. The study methods have been previously described in detail (1, 2). The study was undertaken across two sites: The David Hide Asthma and Allergy Research Centre, St Mary's Hospital, Isle of Wight and the National Institute of Health Research Southampton Biomedical Research Centre based at University Hospital Southampton. The initial study was conducted between January 2011 and October 2014, follow-up between September 2017 and December 2018. Approval for follow-up age six was granted by NRES Committee (Yorkshire and the Humber- Sheffield Research Ethics Committee, IRAS project ID: 204152, REC reference: 16/YH/0217). Participants' parents provided written informed consent. The trial has been registered on ClinicalTrials.gov, ID NCT03763630.

Participants

Infants aged between five and nine months were recruited into the MAPS cohort at primary care child health clinics. Inclusion criteria were a strong family history of atopy (two or more first-degree relatives with allergic disease [food allergy, asthma, eczema, allergic rhinoconjunctivitis]) and negative skin-prick test (SPT) to HDM, cat, grass pollen, peanut, egg and milk (ALK-Abelló, Hørsholm, Denmark). In parallel to MAPS, antenatal recruitment occurred into the observational Immune Tolerance in Early Childhood (ITEC) cohort. Each participant could only belong to one cohort. Some participants, recruited at birth into ITEC, opted to join the MAPS study age 6 months. They were therefore considered MAPS participants and no longer part of the ITEC cohort (and analysed as MAPS). MAPS participants were reviewed at 9, 12, 15, 18, 36 and 72 months of age while the ITEC participants were seen at 5, 18, 36 and 72 months of age with similar assessment (see Figure S1).

Sample Size

The sample size required to detect correct ordering of the primary outcomes (sensitization) between the two groups for the previous proof of efficacy study was calculated as 60 subjects per group, with 85% probability of demonstrating correct ordering of the groups. This was based on a sensitization rate of 0.27 in the placebo group, as observed at age two years in previous studies. (2, 3) The study was not powered to detect differences in clinical end-points.

Randomization and masking

Participants were enrolled by the study team and randomized 1:1 in computer-generated blocks by ALK-Abelló with participants, care providers and all members of study team remaining blinded to group allocation. Investigational medicinal product (IMP) arrived pre-randomized, received by the pharmacy department and was sequentially allocated to participants.

Intervention

In the MAPS intervention group, participants received 2000 standard treatment units of glycerinated HDM allergen extract (ALK-Abelló) per day equal to 11 µg of HDM allergen (equal parts of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*). There was a two-third to one-third mix of group 1 and 2 HDM allergens in a 50% glycerine solution. The placebo was a 50% glycerine solution plus normal saline.. Both products were formulated as oral drops.

Procedures

Study Questionnaires: Participants underwent assessment using the validated International Study of Asthma and Allergies in Childhood questionnaires, study-specific questionnaires and SCORAD forms to assess presence of wheeze, food allergy and eczema severity.

Skin-Prick Tests (SPT): SPTs to common aeroallergens and food allergens were performed using standardized reagents from ALK-Abelló, Hørsholm, Denmark. Mean wheal diameter was calculated. A positive response was defined as a mean wheal diameter ≥ 3 mm than that elicited by the negative control. At all study visits SPTs were carried out to HDM, grass pollen, cat, peanut, milk and egg. From age three years, additional SPT were performed to tree pollen, alternaria and dog.

Fractional exhaled Nitric Oxide (FeNO): FeNO levels were measured at age six only, using the single expiratory breath method with a chemiluminescence analyser (Niox Vero desktop system, Aerocrine, Solna, Sweden). FeNO measurement was performed as recommended by the ATS/ERS guidelines (4). A biofeedback video incentive was used to maintain the expiratory flow rate at 50 ml/s and subjects exhale against resistance to prevent upper airway contamination. Measurements were made in a standardised manner with the subject standing without a nose clip and repeated until two consecutive results within 10% or 5ppb were obtained; with a maximum of eight attempts. All measurements were undertaken before spirometry testing.

Incentive Spirometry and Reversibility: Incentive spirometry was performed at age six, using the Koko system (nSpire Health, Longmont, USA). Forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), mid expiratory flow (MEF), peak expiratory flow (PEF) were recorded. Percent predicted values for age, height, sex and ethnic origin were calculated for the above data, along with forced expiratory ratio (FEV_1/FVC). Spirometry was then repeated, where possible, 15 minutes after administration of 600mcg of salbutamol via large volume spacer. Reversibility was defined as $\geq 12\%$ increase in FEV_1 . Spirometry was performed according to the American Thoracic Society / European Respiratory Society guidelines. (5) To perform the test participants had to be free from respiratory infection for the past 14 days, and have abstained from short acting beta-2-agonists for 6 hours, long acting beta-2-agonists for 12 hours. To ensure validity and reproducibility three technically

acceptable blows with a maximum of 8 attempts were obtained. The highest of two FEV₁ measurements within 10% of each were used.

Methacholine bronchial hyper-responsiveness testing (BHR): Methacholine challenge was used to assess bronchial hyperresponsiveness at age six. Testing was performed using a variation of the standardized international protocol recommended by the European Respiratory Society (6). Trained, experienced research nurses undertook the procedure with direct medical supervision. A pre-test spirometry reading was obtained to ensure an FEV₁ of above 75% predicted for age and height. Initial inhalation of 0.9% saline was followed 30 and 90 seconds later by spirometry recording to obtain a baseline value. Subsequently, incremental doses from 1.4 µg to 716.8 µg of methacholine were serially administered at five minute intervals, according to the Peter Sterk PD₂₀ protocol (6), using a computerized dosimeter system (Koko Digidoser, PDS Instrumentation, Louisville, USA). To perform this test, participants were required to be free from respiratory infection for 14 days, not taking a course of oral steroids, not taken short acting beta-2-agonist for 6 hours, and long acting beta-2-agonist for 12 hours, and having abstained from caffeine intake for at least 4 hours. The dose of methacholine used was recorded whether it produced a 20% fall in FEV₁ or not. At the completion of the protocol or following a 20% fall in FEV₁, 600mcg of salbutamol was administered by large volume spacer. After 15 minutes FEV₁ was measured one final time, and the procedure terminated once FEV₁ returned to 90% or above of baseline.

The methacholine concentration causing a 20% fall in FEV₁ from the post-saline value was interpolated and expressed as PD₂₀ FEV₁ (provocative dose causing a 20% fall in FEV₁). Bronchial responsiveness was defined in relation to the methacholine PD₂₀ FEV₁ following guidelines: normal, > 400 µg; borderline, 100-400 µg; mild, 25-100 µg; moderate, 6-25 µg; severe, <6 µg.

Primary and Secondary Outcomes:

Asthma: For the primary outcome of asthma, all cases were reviewed (blindly to the group allocation for MAPS children) by an adjudication committee of three independent paediatric clinicians with expertise in asthma. The information used included symptom history, response to medication, spirometry, reversibility, FeNO and BHR and a consensus clinical decision was made as to whether or not individual participants had asthma. Participants were allocated to one of five categories: definite asthma, probable asthma, possible asthma, probable not asthma and no asthma.

- “Definite asthma” – where the diagnosis was unquestioned, for example a child with multiple wheezing episodes in the past year, on multiple inhalers with response to these and, where available, abnormal lung function.
- “Probable asthma”- where asthma was the most probable diagnosis but couldn’t be confirmed with absolute certainty (for example children with a history of wheezing, on asthma inhalers with good response to these but normal lung function).
- “Possible asthma” – where there was insufficient information to either confirm or reject the diagnosis.
- “Probable not asthma”- where asthma was highly unlikely based on information provided, but couldn’t be completely rejected as a diagnosis (for example children with no history of symptoms and treatment but with unexpected bronchial hyperactivity in the moderate category).
- “Definite not asthma”- where there was no indication of asthma.

For the purpose of analysis, the “definite not asthma” and “probable not asthma” were grouped together. Comparisons were made between “Definite asthma” cases and “likely asthma (definite + probable asthma) cases.

Secondary outcomes included cumulative sensitization to any tested allergen, cumulative sensitization to HDM, BHR, FeNO, allergic rhinitis, wheeze, eczema and food allergy.

Wheeze and recurrent toddler wheeze: Wheeze was reported by parents. Recurrent wheeze was defined as the presence of three or more separate wheezing episodes before the age of four.

Eczema: Diagnosis of eczema was based at each assessment on the presence of three of the major diagnostic criteria as defined by Hanifin and Rajka- an itchy rash; in a typical distribution (elbow creases, behind knees, face in an infant) that had persisted for more than 7 days and was coming and going for 6 months or more to indicate it's chronic and relapsing nature. (7) Diagnosis was based on questionnaire response as most children had quiescent disease at assessment and therefore SCORAD scores were generally very low. (8)

Rhinitis: Parental report of a sneezy, runny or blocked nose not associated with a cough/ cold as based on questionnaire response at six years of age.

Food allergy: Food allergy was defined as parental report of two or more typical type I hypersensitivity symptoms (urticaria, angioedema, wheeze, diarrhoea and vomiting, oral symptoms, systemic upset) within two hours of ingestion of the index food (a known allergic food). Where possible results were correlated to SPT results (for egg, milk and peanut) to confirm the diagnosis. All possible cases of food reaction were individually reviewed.

Statistical analysis:

The study was designed as proof-of-concept to detect hypothesized correct ordering of sensitization age 18 months, and was not powered to detect differences in clinical end-points. An intention-to-treat analysis was performed for all participants that attended at least one post-randomization assessment. No data was censored or imputed. The analysis assumed that any missing data is missing at random. Differences in proportions of participants between the two groups were calculated with regards to primary and secondary end-points, with 95% CI, using appropriate statistics. Time-to-event analysis was undertaken to compare the proportions of participants sensitized in the two groups over time, and Kaplan-Meier curves were generated. The ITEC cohort represents participants who were at high risk for developing allergy (as per MAPS intervention trial) who did not enrol in the

MAPS study. A comparison of the baseline characteristics of the ITEC group and the MAPS control group was made. This was to ensure that the results were not biased by factors affecting families' willingness to participated in the MAPS study. As this was not the case, the above analysis was repeated with comparison between the MAPS intervention group and a combination of the MAPS placebo and ITEC group providing improved power to assess impact of the MAPS intervention. A sensitivity analysis was performed without ITEC participants found to be sensitized at five months of age. Data was initially collected onto paper questionnaire and clinical record forms (CRF) and then doubly entered into the study database, either a community version of Open Clinical database hosted by University Hospital Southampton NHS Foundation Trust for the three year data, or a Microsoft Access database hosted by the Isle of Wight NHS Trust for the six year data. Data management was under the supervision of the Clinical Trials Manager in the David Hide Asthma and Allergy Research Centre, Isle of Wight. Prior to any statistical analysis, all variables were checked for number of missing values, impossible and improbable values. Impossible and improbable values were defined by clinical opinion. Improbable values also included values that were outside three standard deviations of the mean value, any questions regarding the data were queried with the Data Manger. Descriptive statistics were calculated for all variables and distributional assumptions checked.

RESULTS

Lung function age 6 years

67 participants (78.8%) underwent baseline spirometry, 33 (80.5%) in the intervention group and 34 (77.3%) in the placebo group. There was no statistical significant difference between the two groups, with the intervention group having a trend for lower baseline FEV₁ (99.9% predicted versus 106.4% predicted in the placebo group [6.5% difference, 95% CI -15.1%, 2.1%, $p=0.13$]) but also less reversibility following salbutamol administration (2.8% absolute improvement compared to 4.4% in the control group [1.6% difference, 95% CI -5.1, 1.9, $p=0.36$]).

Exhaled nitric oxide (FeNO) was measured in 47 (55.3%) of participants, 25 in the intervention group and 22 in the placebo group. Median value for the active group was 7.5 ppb (IQR 6.5- 10.5); median value for placebo group 8.75 ppb (6.5-10.5). ($p=0.65$) (Fig S3). One participant in each group had an average FeNO result of >20 ppb.

Bronchial hyper-responsiveness (BHR) was assessed by methacholine challenge in 41 participants (48.2%), 22 in the intervention group and 19 in the placebo group. PD₂₀ values (dose of methacholine resulting in a 20% decrease in FEV₁) were calculated as suggested by Coates et al (6) based on the FEV₁ of the last and previous to last doses. Based on these results participants were categorized into levels of bronchial hyper-responsiveness: severe (PD₂₀ <6), moderate (PD₂₀ 7-25), mild (PD₂₀ 26-100), borderline (PD₂₀ 101-400) and normal (PD₂₀ >400). There was no overall significant difference in the PD₂₀ results between the group ($p=0.79$, Chi-squared test for trend); however 1 (4.6%) of the intervention group had moderate/severe BHR on testing compared to 3 (15.8%) of the placebo group (Table S3).

Diagnosis of asthma is challenging especially in young children with overlapping diagnoses. Multiple guidelines agree that a diagnosis based on symptoms alone is inadequate, and

where possible objective lung function testing should be performed.(9, 10). However, an abnormal lung function or reversibility could not be an essential criteria in the presence of asthma treatment. To overcome these conflicting requirements, a panel of independent paediatric respiratory physicians (blind to the group allocation) reviewed all data available for participants including symptoms, asthma treatment and objective parameters (spirometry, reversibility, FeNO and BHR), to reach a consensus decision regarding asthma status. This approach to asthma diagnosis is supported by evidence that in research studies a definition of childhood asthma should be based on a composite score of reported medical diagnosis, symptoms and lung function (11). Participants were classified into five groups based on the probability of asthma. A comparison of differences between the five groups for baseline FEV₁, reversibility post bronchodilator, FeNO and bronchial hyper-activity demonstrated a trend for greater reversibility, higher median FeNO measurements and more bronchial hyper-responsiveness in the asthma/ probable asthma groups (Figure S4). Participants with “possible asthma” were excluded from analysis, to prevent incorrect group assignment.

Sensitivity analysis

A sensitivity analysis was carried out excluding the ITEC participants who were sensitized on SPT at the initial assessment age six months. Exclusion of these 12 participants did not alter results, and rates of clinical asthma remained significantly higher within the control group compared to intervention group (Table S6).

Figure S1 Overview of the MAPS and ITEC cohorts' study design and follow-up. Follow-up time points were the same for both groups, although the MAPS participants had additional study visits at 9, 12 and 15 months of age whilst receiving the intervention.

Figure S2 Consort figures for the MAPS (A) and ITEC (B) study population. The intention-to-treat analysis included all participants with at least one follow-up visit.

Figure S3 Participant average FeNO results sorted by group. Median value for SLIT group = 7.5 ppb (IQR 6.5- 10.5); median value for placebo group 8.75 ppb (6.5-10.5). ($p=0.65$, Mann Whitney-U test.)

Figure S4 Comparison between five asthma categories of: A - FEV₁ % predicted values (mean and 95% C.I.); B- absolute % change in FEV₁ post bronchodilator (mean and 95% C.I.); C – exhaled nitric oxide values (median and IQR); D – bronchial hyper-responsiveness category proportion. Results shown for the MAPS cohort alone and MAPS and ITEC cohort combined.

Table S1 Baseline characteristics for participants assessed at the different time-points. Atopy defined as sensitization based on positive SPT to any allergen, parental atopic disease defined as parental report of asthma, eczema, hayfever or food allergy. Numbers represent frequencies (percentage) or mean (S.D.). MAPS= Mite Allergy Prevention Study participants (intervention cohort). ITEC= Immune Tolerance in Early Childhood participants (observational high risk cohort).

	MAPS HDM SLIT – baseline (N= 57)	MAPS placebo – baseline (N= 54)	ITEC cohort (N= 224)	MAPS HDM SLIT- 6 years (N=41)	MAPS - placebo- 6 years (N= 44)	ITEC- 6 years (N= 142)
Male	30 (52.6%)	27 (50.0%)	115 (51.3%)	22 (53.7%)	18 (40.9%)	72 (50.7%)
Age (baseline-months; 6 years- years)	6.7 (1.3)	6.9 (1.2)	6.0 (1.0)	6.9 (0.5)	6.9 (0.45)	7.7 (0.52)
Height (cm)	68.0 (4.2)	68.2 (3.2)	67.2 (3.0)	123.6 (6.0)	121.6 (4.7)	126.3 (6.5)
Weight (kg)	8.2 (1.1)	8.2 (1.0)	7.9 (1.1)	24.3 (3.7)	23.7 (3.0)	26.6 (5.4)
Ethnicity (Caucasian)	48 (94.1%)	46 (97.9%)	198 (97.1%)	34 (91.9%)	38 (97.4%)	139 (97.9%)
Eczema	3 (5.3%)	3 (5.6%)	18 (8.8%)	15 (36.6%)	15 (34.1%)	44 (31.9%)
Reported wheeze	18 (31.6%)	14 (25.9%)	71 (34.6%)	27 (65.9%)	26 (59.1%)	97 (68.3%)
Atopy	0 (0.0%)	0 (0.0%)	14 (6.2%)	12 (29.3%)	12 (27.3%)	60 (42.2%)
Breastfeeding						
Never breastfed	6 (10.5%)	5 (9.3%)	35 (17.3%)	4 (9.8%)	4 (9.1%)	19 (15.5%)
Breastfed <2 months	21 (36.8%)	14 (25.9%)	69 (34.2%)	14 (34.2%)	12 (27.3%)	41 (33.3%)
Breastfed 2-4 months	9 (15.8%)	6 (11.1%)	19 (9.4%)	6 (14.6%)	4 (9.1%)	8 (6.5%)
Breastfed > 4 months	21 (36.8%)	29 (53.7%)	79 (39.1%)	17 (41.5%)	24 (54.6%)	55 (44.7%)
Pet ownership						
Any	33 (57.9%)	24 (44.4%)	117 (57.1%)	29 (70.7%)	29 (65.9%)	96 (67.6%)
Dog	18 (31.6%)	10 (18.5%)	69 (34.2%)	16 (39.0%)	14 (31.8%)	57 (40.1%)
Cat	18 (31.6%)	11 (20.4%)	63 (28.1%)	16 (39.0%)	20 (45.4%)	35 (24.6%)

Smoking exposure						
No exposure	18 (35.3%)	15 (30.6%)	64 (31.1%)	18 (48.7%)	15 (37.5%)	64 (52.0%)
In utero only	7 (13.7%)	10 (20.4%)	7 (3.4%)	7 (19.0%)	10 (25%)	7 (5.7%)
Postnatally only	10 (19.6%)	6 (12.2%)	75 (36.4%)	3 (8.1%)	2 (5.0%)	23 (18.7%)
In utero and post-natal exposure	16 (31.4%)	18 (36.7%)	60 (29.3%)	9 (24.2%)	13 (32.5%)	29 (23.6%)
Daycare attendance before age 2	30 (52.6%)	26 (50.0%)	77 (38.9%)	25 (51.0%)	22 (50.0%)	53 (44.5%)
Maternal Education level						
Educated to age 16 years	9 (19.6%)	12 (26.7%)	60 (24.5%)	7 (20.6%)	8 (21.6%)	32 (26.2%)
Educated to age 18 years	17 (37.0%)	18 (40.0%)	113 (46.1%)	12 (35.3%)	17 (46.0%)	57 (46.7%)
Higher education after 18 years	20 (43.5%)	15 (33.3%)	72 (29.4%)	15 (44.1%)	12 (32.4%)	33 (25.1%)
Maternal atopic disease	49 (86.0%)	46 (85.2%)	186 (90.3%)	36 (87.8%)	39 (88.6%)	109 (88.6%)
Paternal atopic disease	46 (90.2%)	42 (87.5%)	167 (81.5%)	34 (91.8%)	33 (84.6%)	97 (78.9%)

Table S2 ITEC Cohort comparison. Comparison of baseline characteristics between the MAPS placebo control group and the ITEC cohort. Numbers represent frequencies (percentages). *numbers represent means (S.D.) **Chi-squared test for trend. $p < 0.05$ deemed statistically significant.

	MAPS Control (N=54)	ITEC control (N=224)	Difference (95% CI) (p value)
Sex (female)	27 (50.0 %)	109 (48.7%)	0.01 (-0.16, 0.14) (0.860)
Birth-weight (grams)*	3397 (477)	3519 (499)	122 (-195, 440) (0.45)
Parental atopy- (both parents atopic)	39 (81.3%)	148 (72.6%)	0.09 (-0.04, 0.21) (0.21)
Ethnicity - caucasian	46 (97.87%)	198 (97.06%)	0.01 (-0.06, 0.04) (0.760)
Maternal education • Educated to age 16 • Educated to age 18 • Higher education	12 (26.67%) 18 (40.0%) 15 (33.3%)	54 (26.6%) 92 (45.32%) 57 (28.08%)	p = 0.668**
Age at 6 month assessment (months)*	6.81 (1.10)	6.03 (0.96)	0.79 (1.09- 0.49) (<0.001)
Height 6 months (cm)*	68.09 (3.10)	67.22 (3.00)	0.86 (-1.79, 0.07) (0.071)
Weight 6 months (kg)*	8.18 (1.01)	7.91 (1.11)	0.27 (-0.06, 0.6) (0.113)
Breastfeeding • Never breastfed • <2 months • 2-4 months • >4 months	5 (9.3%) 14 (25.9%) 6 (11.1%) 29 (53.7%)	35 (17.3%) 69 (34.2%) 19 (9.4%) 79 (39.1%)	P= 0.028**
Pet at home early life (yes)	24 (46.2%)	117 (57.1%)	0.11 (-0.04, 0.26) (0.158)
Dog at home early life (yes)	15 (27.78%)	69 (30.8%)	0.03 (-0.10, 0.16) (0.664)
Cat at home early life (yes)	16 (29.63%)	63 (28.13%)	-.02 (-0.15, 0.12) (0.826)
Smoke exposure (6 months) • No exposure • In utero or childhood exposure only • In utero and childhood exposure	16 (32.7%) 15 (30.6%) 18 (36.7%)	63 (30.7%) 82 (40.0%) 60 (29.3%)	P = 0.706**
Eczema age 6 months	5 (5.56%)	18 (8.78%)	0.03 (-0.04, 0.10) (0.44)
Atopy age 6 months	0 (0.0)	12 (5.36)	0.05 (0.02, 0.08) (0.082)

Table S3 BHR (bronchial hyper-responsiveness) range distribution within each group. ($p=0.79$, Chi-squared test for trend). BHR category defined based on PD20 values - severe (PD20 <6), moderate (PD20 7-25), mild (PD20 26-100), borderline (PD20 101-400) and normal (PD20>400).

BHR category	HDM SLIT (N=22)	Placebo (N=19)	P Value
Severe	0 (0.0%)	2 (10.5%)	0.79
Moderate	1 (4.6%)	1 (5.3%)	
Mild	10 (45.5%)	7 (36.8%)	
Borderline	10 (45.5%)	15 (26.3%)	
Normal	1 (4.6%)	4 (21.1%)	

Table S4 Differences in the proportions of participants with clinical atopic disease by treatment group, age six. Figures represent frequencies (percentages). Differences (95% CIs) represent the difference in proportion between the two groups.

Outcome	HDM SLIT (n = 41)	Placebo (n = 44)	Difference in proportion (95% CI)	P value
Eczema	15 (36.6%)	15 (34.1%)	0.03 (-0.18, 0.23)	0.81
“Ever had” wheeze	27 (65.8%)	26 (59.1%)	0.07 (-0.14, 0.27)	0.52
Recurrent wheeze as toddler	13 (31.7%)	13 (29.6%)	0.02 (-0.17, 0.21)	0.83
Rhinitis	21 (51.3%)	17 (38.6%)	0.13 (-0.08, 0.34)	0.25
Likely food allergy	6 (14.6%)	8 (18.2%)	-0.04 (-0.19, 0.12)	0.66

Table S5 Sensitization rates to individual allergens over the entire study period to six years of age for the MAPS SLIT and placebo groups. Numbers represent frequencies (percentages). Skin prick testing data.

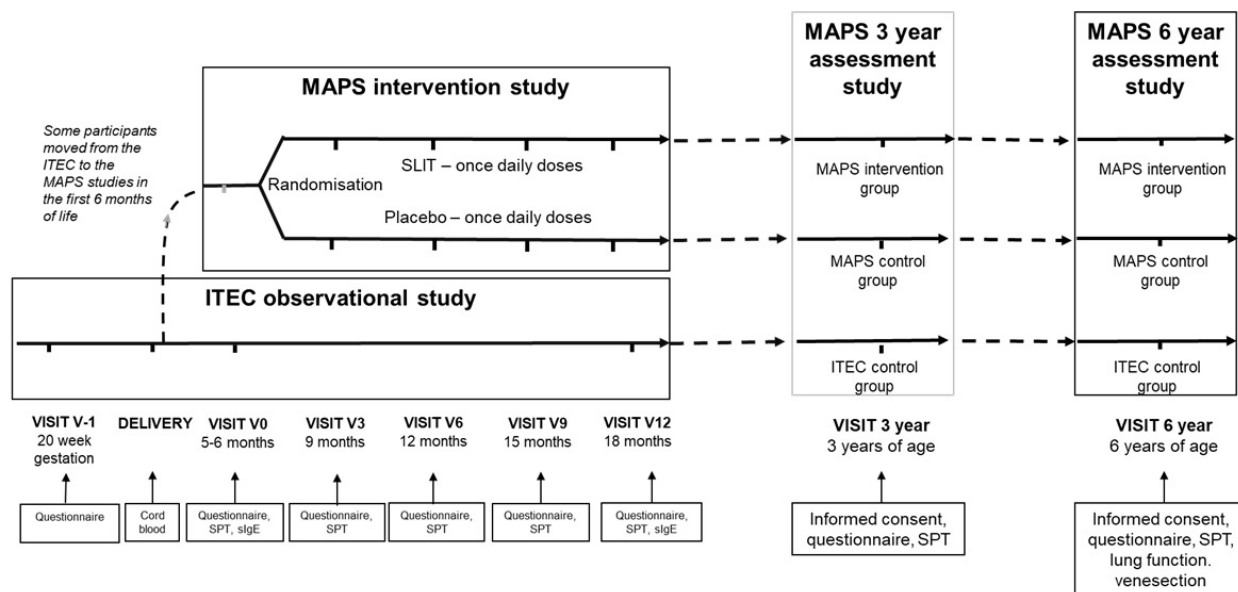
	MAPS SLIT (N= 57)	MAPS placebo (N= 54)
HDM	8 (14.0%)	10 (18.5%)
Grass pollen	8 (14.0%)	7 (13.0%)
Tree pollen	2 (3.5%)	1 (1.9%)
Alternaria	3 (5.3%)	1 (1.9%)
Cat	1 (1.8%)	5 (9.3%)
Dog	4 (7.0%)	3 (5.6%)
Milk	1 (1.8%)	0 (0.0%)
Hen’s egg	2 (3.5%)	2 (3.7%)
Peanut	1 (1.8%)	4 (7.4%)

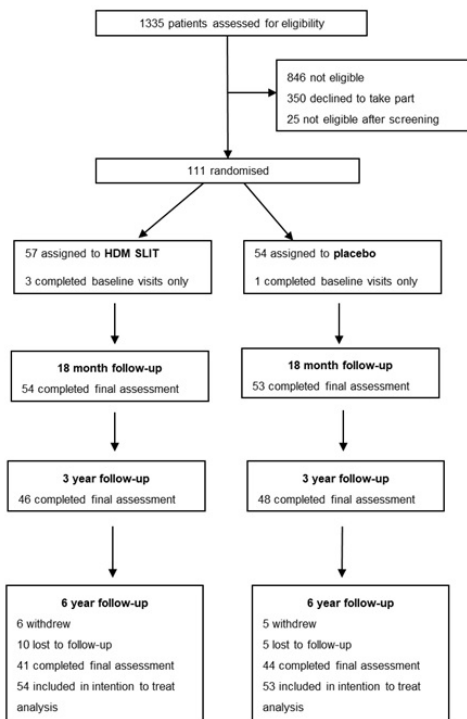
Table S6 Differences in the proportions of participants with asthma and likely asthma (definite asthma + probable asthma) between the MAPS SLIT group and the MAPS placebo + ITEC cohort. 14 ITEC participants who were found to be sensitized at baseline were excluded from this analysis. Figures represent frequencies (percentages). Differences (95% CIs) represent the difference in proportion between the two groups.

Outcome	HDM SLIT (n = 34)	Placebo (n= 156)	Difference in proportion (95% CI)	P value
Asthma	1 (2·9)	23 (15·8)	-0·13 (-0·21, 0·05)	0·048
Likely asthma	4 (10·8)	38 (23·6)	-0·13 (-0·25, -0·01)	0·086

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A MAPS Cohort**B ITEC Cohort**