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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Follow-up, 18 months off house dust mite immunotherapy, of a randomized controlled study on the primary prevention of atopy

To the Editor,

Asthma and other atopic diseases have increased in prevalence over the past 30 years, resulting in a significant socioeconomic impact. Asthma affects up to 10% of children within the United States and is associated with over \$55 billion in annual healthcare costs.¹ Early life allergic sensitization carries the greatest risk for the development of atopic asthma, and primary prevention must therefore target infancy, prior to sensitization occurring. Evidence has emerged that exposure to a high dose of allergen early in life can drive the developing immune system towards a state of tolerance and reduce sensitization,² counteracting the emerging T-helper type 2 lymphocyte (TH2) bias within the developing immune system of those at risk of atopy.

One such strong immune stimulus is house dust mite (HDM), a potent and prevalent allergen. We previously reported the first primary prevention study in infancy using HDM immunotherapy to prevent allergic sensitization.³ This was a double-blind placebo-controlled proof-of-concept study demonstrating that after a year of sub-lingual HDM immunotherapy (SLIT) there was a reduction in sensitization to HDM and other common allergens. The treatment was safe and acceptable to families. Here, we report further follow-up of this cohort at 3 years of age, 18 months off treatment.

The study was carried out as described in Appendix S1. Briefly, 111 infants aged 5-9 months at high risk of atopy (≥ 2 first-degree atopic family members) but with negative skin-prick tests (SPT) were recruited and randomized to either oral allergen extract of HDM or saline placebo twice daily for 12 months. Study visits occurred every 3 months until 18 months of age, then again at 3 years. At the 3-year follow-up, SPTs to HDM, grass pollen, cat, peanut, milk and egg were repeated. Mean wheal diameter was regarded as positive when 3 mm or larger than negative control. Participants also underwent assessment using the validated International Study of Asthma and Allergies in Childhood and study-specific questionnaires and

SCORAD to assess the presence of wheeze, food allergy and eczema severity. An intention to treat analysis was undertaken using Stata software, version 14 (StataCorp, College Station, TX).

A total of 111 infants were randomized into the original study, 57 (51.4%) in the active group and 54 (48.6%) in the placebo group (Appendix S2). The groups were balanced at baseline (Appendix S3) although a higher proportion of the active group had pets and were more likely to have eczema and reported wheeze. A total of 107 participants have at least one follow-up, and 3-year follow-up data were available for 94 (84%) participants, 46 being in the active group.

For the co-primary outcome of sensitization to any allergen (Figure 1A), less children were sensitized in the active group (12 [22.2%]) compared with the control group (18 [34.0%], 11.7% difference [95% CI, -5.1% to 28.6%]), ($P = 0.12$). A similar proportion of children were specifically sensitized to HDM (Figure 1B), 6 (11.1%) in the active group compared to 7 (13.2%) in the control group at 3 years (difference 2.1% [95% CI, -10.3% to 14.5%] $P = 0.8$).

With regard to atopic disease age three, there was no statistically significant difference between the two groups for eczema, "between colds wheeze" or food allergy (Table 1). There was a statistically significant difference ($P = 0.04$) in the proportions that had ever wheezed with more in the active group (26 [56.5%]) compared to the placebo group (17 [35.4%]), amounting to a 21.1% difference in proportion [95% CI, 1%-40%]).

At age three, this primary prevention study demonstrates a maintained trend for reduction in cumulative allergen sensitization amongst infants receiving HDM SLIT compared to controls, as was observed at 18 months. However, this trend no longer reaches statistical significance. This was a proof-of-concept study, powered only to detect correct ordering of the primary outcomes (sensitization), achieved at both 18 months and 3 years.³ No difference was seen in sensitization rate to HDM alone. However, HDM allergen

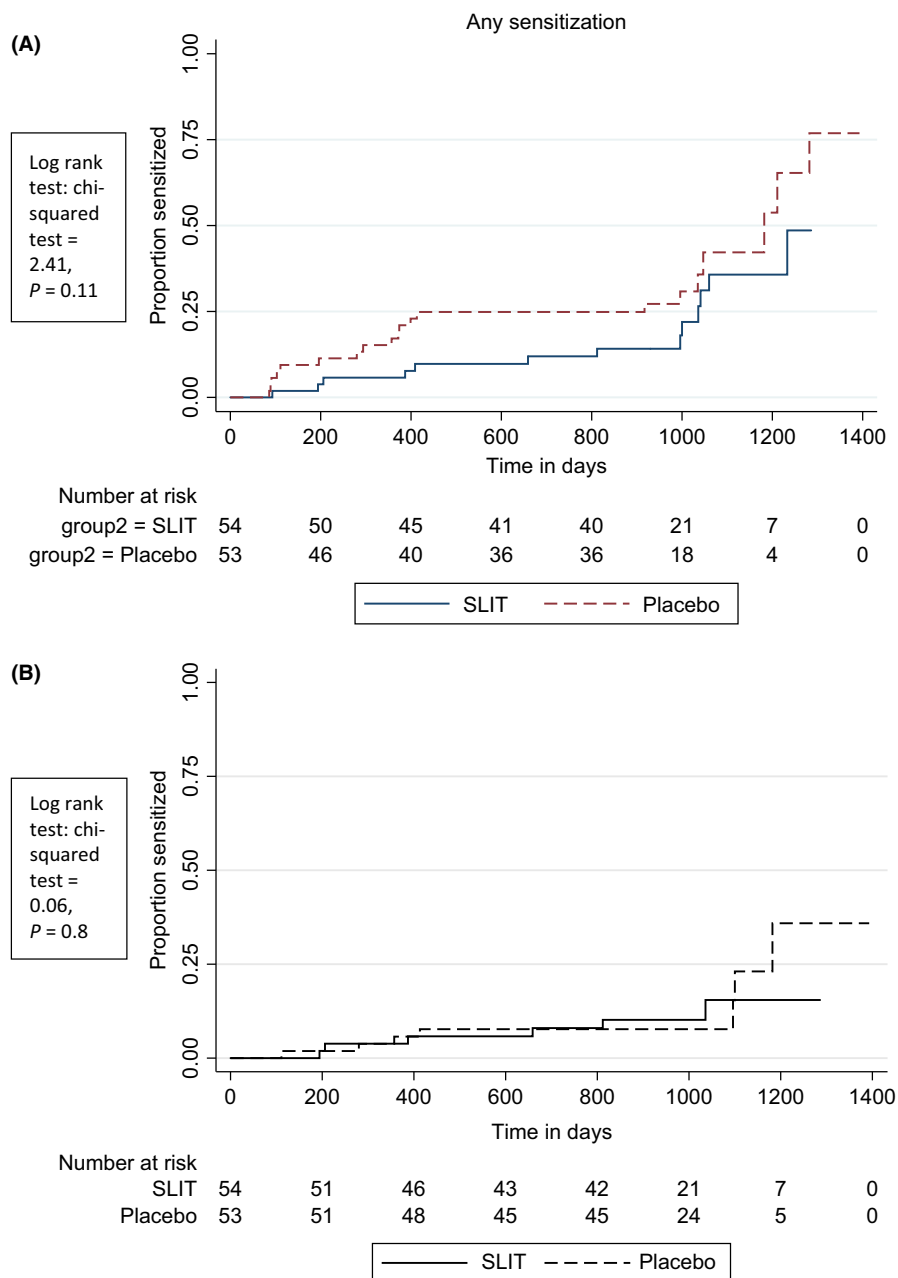


FIGURE 1 Kaplan-Meier plot comparing sub-lingual house dust mite (HDM) immunotherapy and placebo participants' cumulative sensitization to any common allergen (A) and HDM (B) over the follow-up period [Colour figure can be viewed at wileyonlinelibrary.com]

was selected for its ability to modulate the developing immune system through allergenic, endotoxin and enzymatic effects, rather than an allergen-specific effect.⁴ We therefore hypothesized that it would have a bystander effect on sensitization to other allergens. Sensitization to any allergen was therefore a defined co-primary outcome.

At 3 years, we did not observe a significant effect on clinical outcomes between the groups for eczema and food allergies. There was a significant difference in the "ever had" wheeze group, with a higher proportion of the SLIT group having ever wheezed. This could be explained by the higher proportion of active group participants reporting wheeze at baseline (34% vs 25.5%),³ and at age three, a large proportion of wheezing episodes is related to inter-current viral infections. Another possible explanation relates to the high endotoxin content of HDM SLIT, associated with an increased risk of

TABLE 1 Differences in the proportions of participants in clinical outcomes by treatment group

Outcome	HDM OIT (n = 46)	Placebo (n = 48)	Difference in proportion (95% CI)	P value
Eczema	11 (23.9)	10 (20.8)	0.03 (−0.1, 0.2)	0.7
"Ever had" wheeze	26 (56.5)	17 (35.4)	0.2 (0.01, 0.4)	0.04
"Between colds" wheeze	4 (8.7)	2 (4.2)	0.05 (−0.05, 0.1)	0.4
Food allergy	5 (10.9)	6 (12.5)	−0.02 (−0.1, 0.1)	0.8

Figures represent frequencies (percentages). Differences (95% CIs) represent the difference in proportion between the two groups. A positive difference favours the placebo group. Wheeze was reported by parents; eczema was defined as a persistent itchy rash affecting flexural surfaces or the face; food allergy was defined based on the presence of typical symptoms within two hours of target food ingestion.

wheezing in infants and toddlers, but a protective effect on the later asthma development.⁵

Our study was a small, single-centre proof-of-concept study and therefore has a number of limitations. Baseline eczema rates within the study group were much lower than expected from similar high-risk cohorts. Given that early life atopic dermatitis is a strong influencing factor for later allergic sensitization, it would have perhaps been beneficial to recruit more infants with eczema. It may also have been more effective to use a larger allergen dose and longer duration of treatment, although the allergen dose used has previously been shown to induce a significant immunological response.⁶

A recent meta-analysis on the use of AIT as immunoprophylaxis was inconclusive,⁷ due to lack of placebo-controlled studies. Previously conducted studies also focused only on already sensitized older children, with variable results.^{8,9} Our proof-of-concept study continues to demonstrate that prophylactic immunotherapy in early childhood may be effective in preventing later sensitization in this high-risk cohort, an effect that is partially maintained for at least 18 months following cessation of AIT. We have commenced the 6-year assessment of the participants, to evaluate sensitization rates and clinical outcomes. Further, adequately powered, studies are required to assess the efficacy of this approach.

CONFLICTS OF INTEREST

G. Roberts has received research support from the National Institute for Health Research (NIHR), has received lecture fees and intervention and placebo medications from ALK-Abello, is a member of the ALK-Abello advisory board. H. Arshad has received research support from the NIHR, National Institute of Health, US, Medical Research Council UK and ALK-Abello, has received consultancy fees from Merck, and has a patent for the use of house dust mite allergen immunotherapy for primary prevention of asthma and atopy.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix 1. Study methods.

Study design

This was a prospective, randomized, double-blind placebo-controlled phase IIa study. The initial study was conducted between January 2011 and October 2014, follow-up between March 2014 and July 2016 (fig 1). The study methods have been previously described in detail (1). 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 Respiratory Biomedical Research Unit based at University Hospital Southampton. Approval for the initial study was obtained from the Medicines and Healthcare products Regulatory Agency (Eudract no 2009-015679-28) and ethics approval was given by the South Central Ethics Committee (09/H0504/124). Approval for the follow-up at three years was granted by NRES Committee South Central - Hampshire A (IRAS project ID: 138317). Participants' parents provided written informed consent.

Participants

Infants aged between 5 and 9 months were recruited at primary care child health clinics. Inclusion criteria were a strong family history of atopy (≥ 2 first-degree relatives with allergic disease [food allergy, asthma, eczema, rhinoconjunctivitis]) and negative skin-prick test (SPT) to HDM, cat, grass pollen, peanut, egg and milk (ALK-Abell , H rsholm, Denmark).

Intervention

In the active group, participants received 2000 standard treatment units of glycerinated HDM allergen extract (ALK-Abell ) per day. Normal saline was administered to the placebo group. Both products were formulated as oral drops. In the active group the sub-lingual immunotherapy dose equated to 11 μ g of HDM allergen (equal parts of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*). Parents were taught how to administer the HDM SLIT twice daily for the following 12 months.

Randomization

Participants were randomized in computer-generated blocks performed by ALK-Abell . Participants and study team remained blinded to group allocation. Investigational medicinal product (IMP) arrived pre-randomized and was sequentially allocated to participants.

Procedures

SPTs to common aeroallergens and food allergens were performed by research nurses at all time-points using standardized reagents from ALK-Abell . Wheal size diameters were measured by nurses and mean wheal diameter was calculated (longest diameter plus diameter perpendicular to this divided by 2). 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. Participants also underwent assessment using the validated International Study of Asthma and Allergies in Childhood, study-specific questionnaires and SCORAD forms to assess presence of wheeze, food allergy and eczema severity. During the initial study, study visits occurred every 3 months until 18 months of age. This was subsequently followed by a single follow-up visit close to each participant's third birthday.

Primary and secondary outcomes

The *a priori* co-primary outcomes were cumulative sensitization to HDM and sensitization to any tested allergen, assessed by SPTs. Secondary outcomes were prevalence of wheeze, eczema and food allergy. Wheeze was reported by parents, eczema was defined as a persistent (7 day or more) itchy rash affecting the flexural surfaces or the face. Food allergy was defined based on the development of typical symptoms within two hours of ingestion.

Statistical analysis

The sample size required to detect correct ordering of the primary outcomes (sensitization) between the 2 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 2 years in previous studies (1, 2). The study was designed as a proof-of-concept study 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. Time-to-event analysis was undertaken to compare the proportions of participants sensitized in the 2 groups over time, and Kaplan-Meier curves were generated. Differences in proportions of participants with secondary clinical outcomes were calculated with 95% confidence intervals (CI). Statistical analysis was performed with Stata, version 14 (StataCorp, College Station, USA).

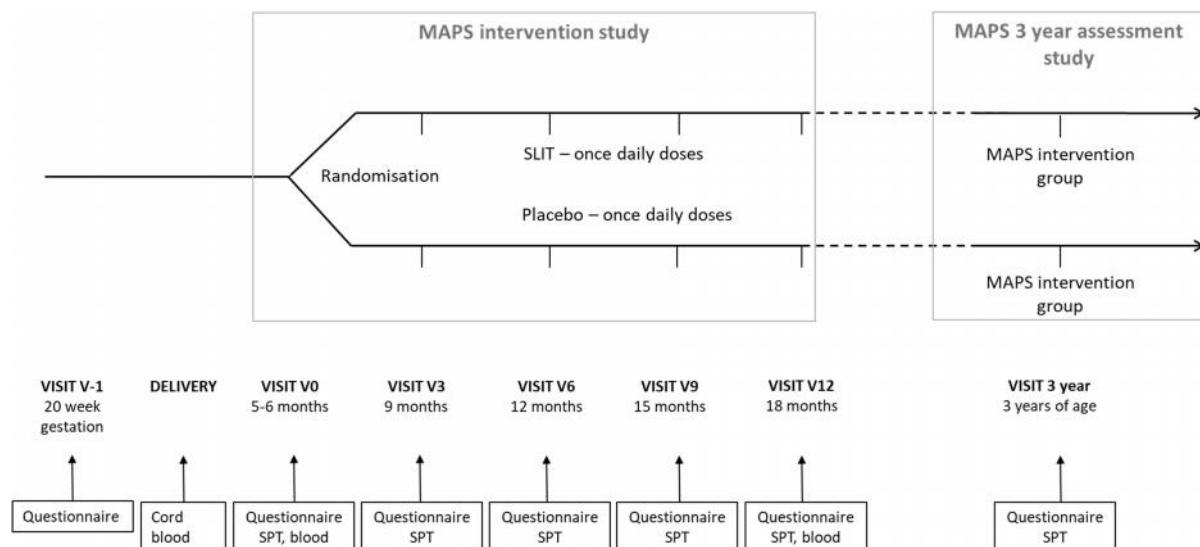


Figure 1. Outline of study design.

Appendix 2. Study participants

A total of 1335 infants were screened; 846 were not eligible and 350 did not wish to take part (Fig 2) 136 infants attended a screening visit, 25 were not eligible and thus 111 were randomized into the study. 57 (51.4%) were assigned to the active group and 54 (48.6%) to the placebo group. 4 in the active group and 3 in the control group only attended the initial screening visit. 3 of these (1 in the active, 2 in the placebo) attended for their 3 year follow-up. Follow-up information for at least one time-point was available for 54 and 53 participants in the active and placebo groups respectively; these were included in the intention-to-treat analysis. A total of 46 participants in the active and 48 participants in the placebo group completed the assessment at three years of age, with respectively 11 and 6 participants being lost to follow-up or withdrawing. Follow-up data at three years was therefor available for 94 (84%) of participants.

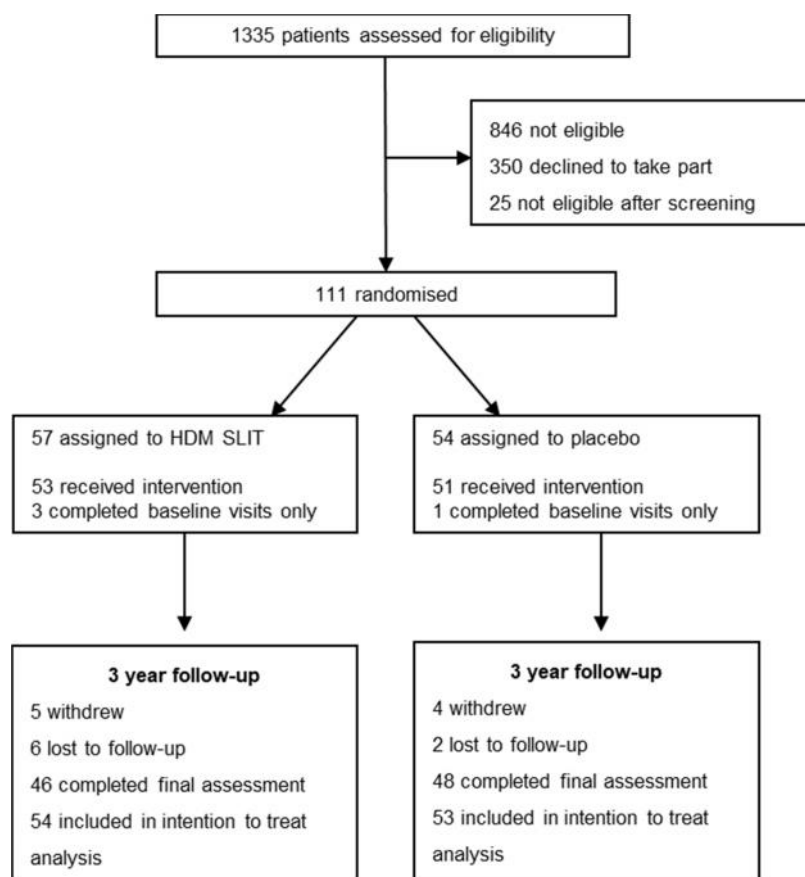


Figure 2. CONSORT flow diagram of the study process. SLIT, sub-lingual immunotherapy.

Appendix 3. Study participants characteristics

Characteristic	HDM SLIT Baseline (N= 53)	HDM SLIT 3 year follow-up (N= 46)	PLACEBO Baseline (N= 51)	PLACEBO 3 year follow-up (N=48)
Male	29 (54.7)	24 (52.2)	24 (47.0)	23 (48.9)
Eczema	8 (15.1)	11 (23.9)	3 (5.9)	10 (20.8)
Reported wheeze	18 (34.0)	26 (56.5)	13 (25.5)	17 (35.4)
Reported allergic reaction to a food	0 (0.0)	5 (10.9)	0 (0.0)	6 (12.5)
Age (months)	6.7 [1.3]	41.0 [4.5]	6.9 [1.2]	40.4 [4.2]
Height (cms)	68.1 [4.2]	99.1 [3.8]	68.2 [3.2]	97.7 [4.6]
Weight (kg)	8.2 [1.1]	15.9 [1.78]	8.2 [1.0]	16.0 [1.95]
Pet ownership				
Any	32 (60.4)	29 (63.0)	22 (43.1)	28 (59.6)
Dog	18 (34.0)	14 (30.4)	10 (19.6)	14 (29.8)
Cat	18 (34.0)	18 (39.1)	10 (19.6)	10 (21.2)
Other	7 (13.2)	8 (17.4)	10 (19.6)	4 (8.5)
Maternal smoking				
Yes, daily	4 (7.7)	2 (4.3)	6 (12.0)	3 (6.4)
Yes, occasionally	0 (0.0)	0 (0.0)	1 (2.0)	3 (6.4)
No	48 (92.3)	44 (95.7)	43 (86.0)	41 (87.2)
Anyone else smoke inside home				
Yes, daily	8 (15.4)	5 (10.9)	4 (8.0)	4 (8.5)
Yes, occasionally	0 (0.0)	4 (8.7)	3 (6.0)	3 (6.4)
No	44 (86.4)	37 (80.4)	43 (86.0)	40 (85.1)
Family				
Attends day care or nursery	10 (18.9)	46 (100)	5 (10.2)	47 (100)
Total family income (£)				
< 12,000	7 (17.7)	7 (23.3)	5 (13.2)	3 (10.3)
12,000- 17,999	4 (9.8)	4 (13.3)	5 (13.2)	4 (13.8)
18,000-29,999	6 (14.6)	7 (23.3)	6 (15.8)	9 (31.0)
30,000-41,999	10 (24.4)	5 (16.7)	13 (34.2)	8 (27.6)
>42,000	14 (34.2)	7 (23.3)	9 (23.7)	3 (10.3)
Mother				
Asthma	26 (55.3)	21 (51.2)	20 (45.5)	20 (47.6)
Hay fever	43 (91.5)	37 (90.2)	39 (86.7)	34 (80.9)
Eczema	24 (51.1)	21 (51.2)	27 (60.0)	24 (57.1)
Father				
Asthma	23 (48.9)	19 (46.3)	24 (51.1)	23 (56.1)
Hay fever	39 (83.0)	33 (80.5)	33 (75.0)	27 (65.8)
Eczema	10 (21.7)	9 (22.0)	16 (36.4)	16 (39.0)

Table 1 .Characteristics of participants receiving HDM SLIT or placebo at baseline (5 months) and at follow-up age 3. Numbers represent frequencies (percentages) or mean [standard deviation] depending on distribution of data.

Appendix 4. Sensitization to other allergens

	SLIT (n = 46)	Placebo (n= 47)
Cat	0 (0.0)	3 (6.4)
Dog	2 (4.3)	2 (4.3)
Alternaria	2 (4.3)	1 (2.1)
Tree and grass	6 (13.0)	6 (12.8)
Egg, milk and peanut	2 (4.3)	2 (4.3)

Table 2- sensitization to other allergens at follow-up age 3. Number (percentage of total)

Sensitization numbers to other allergens were small, and were mostly equal between the two groups. Sensitization rates age 3 to cat were higher in the placebo vs the SLIT group (6.4% versus 0%). Cat ownership at age three was higher in the SLIT group (39.1%) versus control (21.2%). Previous studies have shown a similar inverse relationship between presence of a cat in the home and cat sensitization rates (3), however in a large pooled analysis cat ownership in early life was not found to be associated with later asthma risk (4). There is however some evidence that early life dog exposure reduces asthma risk at school age (5) . Dog ownership and sensitization rates were very similar between our two groups.

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