Content of supplementary material:

page

Supplementary material:

Table s1: Definition of asthma and allergic rhinoconjunctivitis used at 2 and 6 years

	Disease definition in 2yr follow-up analysis[1]	Disease definition used in this 6 year follow up analysis
Asthma (current)	Based on structured interview by paediatrician. "Asthma was defined as at least three episodes of wheezing in the last 12 months combined with treatment by inhaled glucocorticoids, or signs of suspected hyper-reactivity (cough or wheeze at excitement or impaired night sleep) without concurrent upper respiratory infection." [1]	Based on questionnaire data: Answered "yes" to the above question regarding doctor diagnosed asthma and: • In the **last 12 months,** has your child been treated with tables, inhalers or other medications for wheezing, chest tightness or asthma?
ARC (cumulative incidence)	Based on clinical assessment by paediatrician: "The diagnosis of ARC was a clinical decision made by the paediatrician based on a structured medical history and clinical examination." A diagnosis of ARC was assigned if the paediatrician assessed the likelihood of the disease as 8 out of 8 on a visual analogue scale.[1] This scale was not used in the 6 year follow-up and it is therefore necessary to use a different definition.	Based on questionnaire data: Answered "yes" to the question: • Has your child **ever had** hayfever or allergic rhinoconjunctivitis?

ARC: allergic rhinoconjunctivitis

Table s2: Baseline data, characteristics and allergy related disease at 2 years for those who did and did not submit 6 year child health questionnaire.

	Comparison of drop out cases				
	Submitted 6yr questionnaire		Drop-outs		
	N[a]		N[a]		p-value
Baseline data and characteristics					
Age, mother (years), mean ± SD	276	30.2 (4.0)	104	30.1 (4.4)	0.793
Education, mother (yrs), mean ± SD	265	15.2 (2.3)	99	15.3 (2.3)	0.618
Education, father (yrs), mean ± SD	265	14.7 (2.5)	98	14.9 (2.7)	0.576
Birth weight (g), mean ± SD	263	3619 (460)	100	3660 (519)	0.458
Sex (male), n (%)	281	135 (48.0)	103	41 (39.8)	0.151
Premature, n (%)	273	9 (3.3)	101	3 (3.0)	1.000[c]
Siblings, n (%)	281	121 (43.1)	126	48 (38.1)	0.347
Atopy in family, n (%)	281	201 (71.5)	126	99 (78.6)	0.136
Smoking mother, n (%)	281	23 (8.2)	123	12 (9.8)	0.605
Smoking father, n (%)	280	42 (15.0)	121	31 (25.6)	0.011
Breastfed ≥ 3 months, n (%)	250	247 (98.8)	84	79 (94.1)	0.026[c]
At least one pet at home, n (%)	281	74 (26.3)	126	31 (24.6)	0.712
Used antibiotics, n (%)	244	50 (20.5)	76	19 (25.0)	0.404
Fish ≤ 6 mo., n (%)	248	43 (17.3)	80	19 (23.8)	0.203
Vegetables ≤ 6 mo., n (%)	249	144 (57.8)	83	45 (54.2)	0.565
Protocol compliance, n (%)	232	207 (89.2)	66	51 (77.3)	0.012
Allergy related disease at 2 years					
AD (UKWP), n(%)	223	61 (27.4)	55	16 (29.1)	0.797
AD (question), n(%)	231	50 (21.7)	65	11 (16.9)	0.406
ARC, n(%)	230	15 (6.5)	64	7 (10.9)	0.235
Asthma, n(%)	231	10 (4.3)	65	2 (3.1)	1.000[c]
Atopic sensitisation, n(%)	214	32 (15.0)	50	3 (6.0)	0.093[c]

[a]N: number of observed cases for each variable; [b]p-values calculated using t-test for continuous variables and χ^2 for binary variables; [c]Fisher's exact test used to calculate p-values for binary variables with frequency <5 in one or more cell of the contingency table.

Details of multiple imputation model

Multiple imputations by chained equations (MICE) was chosen for the primary intention to treat (ITT) analysis because of the flexibility of this method in the face of a non-monotone missingness in both outcome variables and covariates. The following supplementary material details the missingness in the data, the imputation model (building, procedure and checking) and imputed estimates.

Description of missingness

The missingness followed a non-monotone pattern with both outcome variables and covariates containing missing values. The rate of missingness in each variable ranged from 1.9 % to 67.2 % (Table s4). Only 68 individuals (16.4%) had a complete dataset for variables used in the multiple imputation model. There was no marked difference in the rate of missingness for individual variables between the probiotic and placebo trial arms (data not shown).

Differences in the baseline characteristics between those who did and did not complete the 6 year clinical examination (Table 1 in main article) and or health questionnaire (Table s2 above) are discussed in the main article.

The multiple imputation procedure was conducted under the assumption that the data is missing at random (MAR). Deviations from this assumption are tested in the sensitivity analysis which is described in more detail below.

Imputation model specifications

Due to the high rate of missingness, 100 imputed datasets were created (m=100). A single imputation model was constructed which included all 6 year outcomes. All baseline characteristics and 2 year disease outcomes shown in Table 1 of the main article were considered for inclusion in the imputation model and chosen based on association with disease outcome or missingness at 2 and 6 years.

The inclusion of as many predictors as possible is advised as to "… avoid making incorrect assumptions about the relationships between the variables."[2] However, in the current dataset the majority of covariates considered for inclusion were binary variables, which presented significant non-convergence problems after only a few imputations when all variables were included in all predication equations. This was addressed in the following ways: (a) parsimonious specification of prediction equations with omission of variables that had no or low correlation with the variable to be imputed, (b) inclusion of SPT and sIgE test results as continuous variables and (c) the use of the `augment` option in the `mi impute chained` Stata command.

Imputation procedure

Multiple imputations were created using the `mi impute chained` command in Stata (IC 13.1, StataCorp, Texas, USA). Table s4 provides details regarding the prediction equations for each imputed variable. Binary variables were imputed using logistic regression and continuous variables

were imputed using predictive mean matching (PMM) because of their non-normal distribution. The order of imputation was the default order in Stata – from the variable with least to most missingness.

Imputation model checking

Convergence of the imputation model was assessed prior to imputation using trace plots over 100 iterations. In some variables there appeared to be slight trends in the imputed values in the first 10-20 iterations and thus a more conservative burn-in of 30 iterations was chosen for the final imputation model. Trace plots for each imputed variables from 3 chains were also reviewed over 100 iterations to confirm that the initial values for each imputation did not materially affect the imputed values. No apparent trends were observed for any of the imputed variables and the imputed values oscillated around a common mean after approximately the 20th iteration (graphs not included).

The following variables were computed from imputed variables: cumulative incidence of AD (both questionnaire and UKWP defined), ARC, wheeze and bronchitis at 6 years and allergic sensitisation at 2 and 6 years. All imputed variables and those constructed from imputed variables were compared to the observed estimates of prevalence / cumulative incidence (Table s5). For the non-normally distributed allergy testing variables (continuous), the predictive mean matching (PMM) method produced a distribution of values which was comparable to the observed distribution (data and graphs not included).

Details of Pattern Mixture Methods (PMM) sensitivity analysis

The MNAR sensitivity analysis is included as a recommended part of the intention to treat (ITT) analysis strategy[3] and because of suspicion that the reported data is partially missing not at random (MNAR) .

Method:

The pattern-mixture model (PMM) version of the user written Stata command, `rctmiss`, was used to assess the estimated effect of probiotic supplementation on each outcome variable under a range of hypothetical deviations from the MAR assumption. The effect of probiotics is estimated using multivariable logistic regression for each of the disease outcome and treatment allocation, family history of atopy, sex, presence of siblings, maternal and paternal smoking, antibiotic use before 1 year and a missing indicator variable are covariates. The coefficient of the missing indicator variable is the informative missing parameter, δ, which is an expression of the hypothetical assumptions regarding the association between missingness and the outcomes. This parameter, δ, is the log of "… the ratio of the odds of the outcome among participants with unobserved outcome to the odds of the outcome among observed participants…", which is known as the Informative Missingness Odds Ratios (IMORs) [4, 5]. Under the MAR assumption for binary variables, IMOR = 1 and thus $\ln(\mathrm{IMOR}) = 0$. The range of IMORs selected for the sensitivity analysis is given in Table s6.

Table s4: Predictive equations for imputed covariates and outcome variables.

Imputed variable	Percent missing (%)	Method of imputation	Covariates omitted from the prediction equation[a]
Baseline / characteristics Covariates			
Family history of atopy	1.9	Logistic	
Presence of older siblings	1.9	Logistic	
Paternal smoking	3.4	Logistic	Current questionnaire defined AD at 2 and 6 years, and
Sex	7.5	Logistic	Current questionnaire defined asthma at 6 years.
Antibiotic use in first year	22.9	Logistic	
Protocol compliance	28.2	Logistic, augmented[b]	
Disease outcomes at 2 years			
Cumulative incidence of AD at yrs, questionnaire defined	28.7	Logistic	Current questionnaire defined AD at 2 and 6 years, Current questionnaire defined asthma at 6 years, Parentally reported wheeze and bronchitis at 1, 2 and 6 years and cumulative report of pneumonia
Prevalence of AD at 2yrs, questionnaire defined	28.7	Logistic, augmented[b]	Imputation conducted conditional on the imputed variable for cumulative incidence of questionnaire defined AD at 2 years being positive. All variables were omitted from the prediction equation except: treatment allocation, the baseline/characteristics covariates, cumulative incidence of UKWP defined AD at 2 years and sensitisation tests (sIgE and SPT) at 2 years.
Cumulative incidence of AD at 2yrs, UKWP diagnostic criteria	33.0	Logistic	Current questionnaire defined AD at 2 and 6 years, Current questionnaire defined asthma at 6 years, Parentally reported wheeze and bronchitis at 1, 2 and 6 years and cumulative report of pneumonia
Cumulative incidence of asthma at 2 years	28.4	Logistic, augmented[b]	All variables omitted except: treatment allocation, family history, sex, cumulative incidence of asthma at 6 years, wheeze at 2 and 6 years, bronchitis at 1 year, allergic rhinoconjunctivitis at 6 years.
Cumulative incidence of ARC at 2 years	29.2	Logistic	Current questionnaire defined AD at 2 and 6 years, and Current questionnaire defined asthma at 6 years
Disease outcomes at 6 years			
Cumulative incidence of AD at 6yrs, questionnaire defined	34.0	Logistic	Current questionnaire defined AD at 2 and 6 years, Current questionnaire defined asthma at 6 years, Parentally reported wheeze and bronchitis at 1, 2 and 6 years and cumulative report of pneumonia
Prevalence of AD at 6yrs, questionnaire defined.	34.0	Logistic	Imputation conducted conditional on the imputed variable for cumulative incidence of asthma at 6 years being positive. All variables were omitted from the prediction equation except: treatment allocation, the baseline covariates, current UKWP defined AD at 6 years, cumulative incidence of questionnaire and UKWP defined AD at 2 years and sensitisation tests (sIgE and SPT) at 2 years.

AD: Atopic dermatitis; ARC: allergic rhinoconjunctivitis; sIgE: specific immunoglobulin E; SPT: skin prick test; UKWP: UK Working Party; [a,b] See foot notes at base of table continuation on the following page.

Table s4: (continued) Predictive equations for imputed covariates and outcome variables.

Imputed variable	Percent missing (%)	Method of imputation	Covariates omitted from the prediction equation[a]
Disease outcomes at 6 years (continued)			
Prevalence of AD at 6yrs, UKWP diagnostic criteria	60.7	Logistic, augmented[b]	Current questionnaire defined AD at 2 and 6 years, Current questionnaire defined asthma at 6 years, Parentally reported wheeze and bronchitis at 1, 2 and 6 years and cumulative report of pneumonia
Cumulative incidence of asthma at 6yrs	32.3	Logistic, augmented[b]	All variables omitted except: treatment allocation, family history, sex, cumulative incidence of asthma at 2 years, wheeze at 6 years, bronchitis at 1, 2 or 6 years.
Prevalence of asthma at 6yrs	32.3	Logistic	Imputation conducted conditional on the imputed variable for cumulative incidence of asthma at 6 years being positive. All variables were omitted from the prediction equation except: treatment allocation, family history, sex, cumulative incidence of asthma at 2 year and wheeze at 6 years.
Cumulative incidence of ARC at 6yrs	32.8	Logistic	Current questionnaire defined AD at 2 and 6 years, and Current questionnaire defined asthma at 6 years
Allergy testing at 2 and 6 years			
Maximum SPT wheal size at 2yrs	42.7	PMM	Current questionnaire defined AD at 2 and 6 years, Current questionnaire defined asthma at 6 years, Parentally reported wheeze and bronchitis at 1, 2 and 6 years and cumulative report of pneumonia
Maximum sIgE level at 2 years	54.5	PMM	
Maximum SPT wheal size at 6yrs	67.2	PMM	
Maximum sIgE level at 6yrs	64.6	PMM	
Wheeze, bronchitis and pneumonia at 1, 2 and 6 years			
Wheeze at 1 year	31.6	Logistic	Cumulative incidence of asthma and ARC at 2 and 6 years. All parentally reported wheeze and bronchitis at 1, 2 and 6 years were included as predictors for each other. Atopic dermatitis and sensitisation variable omitted because of low correlation.
Wheeze at 2 years	31.3	Logistic	
Wheeze at 6 years	34.0	Logistic	
Bronchitis at 1 year	34.9	Logistic	
Bronchitis at 2 years	28.4	Logistic	
Bronchitis at 6 years	35.2	Logistic	
Pneumonia (cumulative) at 6 years	32.8	Logistic	Parentally reported wheeze at 2 and 6 years, parentally reported bronchitis at 6 years Cumulative incidence of asthma at 2 and 6 years

AD: Atopic dermatitis; ARC: allergic rhinoconjunctivitis; PMM: predictive mean matching; sIgE: specific immunoglobulin E; SPT: skin prick test; UKWP: UK Working Party;
[a]Certain variables were omitted from individual prediction equations because of no or low correlation with the variable to be imputed and to avoid non-convergence of the imputation model, [b]The `augment` option was utilised in the Stata command, `mi impute chained`, which adds a few observations with very small weightings to avoid perfect prediction.[2]

Table s5: Summary statistics for comparison of imputed covariates and outcomes

	Observed data, % (95% CI)				Imputed estimates, % (95% CI)		
Variable	**N**	**Total population**	**Probiotic**	**Placebo**	**Total population**	**Probiotic (n=211)**	**Placebo (n=204)**
Auxiliary variables							
Family history	407	73.7 (69.2-77.8)	73.4 (67.0-79.0)	74.0 (67.4-79.6)	73.7 (69.4-78.0)	73.4 (67.3-79.4)	74.0 (67.8-80.1)
Siblings	407	41.5 (36.8-46.4)	44.0 (37.3-50.8)	39.0 (32.5-46.0)	41.5 (36.7-46.3)	44.1 (37.2-50.9)	38.9 (32.1-45.8)
Paternal smoking	401	18.2 (14.7-22.3)	17.2 (12.6-23.1)	19.2 (14.3-25.3)	18.3 (14.5-22.1)	17.4 (12.1-22.6)	19.3 (13.8-24.9)
Sex, male	384	45.8 (40.9-50.9)	49.7 (42.7-56.8)	41.9 (35.1-49.0)	45.9 (40.9-50.9)	49.6 (42.5-56.7)	42.0 (35.0-49.1)
Antibiotic use	320	21.6 (17.4-26.4)	21.7 (16.0-28.8)	21.4 (15.7-28.5)	22.5 (17.8-27.2)	23.7 (17.1-30.2)	21.3 (14.9-27.8)
Compliance	298	86.6 (82.2-90.0)	86.7 (80.2-91.3)	86.5 (79.9-91.1)	84.3 (80.0-88.5)	84.2 (78.1-90.2)	84.4 (78.1-90.6)
2 year outcomes							
Current disease							
AD (question.[a])	296	18.2 (14.2-23.1)	18.1 (12.7-25.2)	18.4 (12.9-25.5)	18.4 (13.9-22.8)	18.1 (12.4-25.3)	17.8 (11.7-24.0)
Sensitisation	264	13.3 (9.7-17.9)	15.3 (10.0-22.6)	11.3 (6.9-18.0)	18.6 (13.0-24.2)	20.8 (13.1-28.3)	16.3 (9.3-23.4)
Cumulative incidence							
AD (UKWP)	278	27.7 (22.7-33.3)	21.0 (15.0-28.7)	34.3 (26.8-42.6)	29.1 (23.7-34.5)	23.7 (16.6-30.7)	34.6 (26.9-42.3)
AD (question.)	296	20.6 (16.4-25.6)	21.5 (15.6-28.9)	19.7 (14.0-27.0)	21.8 (17.2-26.4)	23.8 (17.0-30.6)	19.7 (13.5-25.9)
ARC	294	7.5 (5.0-11.1)	7.5 (4.2-13.1)	7.4 (4.1-13.0)	10.0 (6.1-13.9)	10.5 (4.9-16.2)	9.4 (4.2-14.5)
Asthma	297	4.0 (2.3-7.0)	3.4 (1.4-7.9)	4.7 (2.3-9.6)	5.2 (2.5-8.0)	4.6 (0.8-8.5)	5.8 (1.9-9.8)
6 year outcomes							
Current disease							
AD (question.)	274	15.7 (11.8-20.5)	15.2 (10.0-22.4)	16.2 (11.0-23.3)	16.8 (12.2-21.4)	17.0 (10.0-23.9)	16.6 (10.5-22.8)
AD (UKWP)	163	16.0 (11.1-22.5)	14.8 (8.5-24.5)	17.1 (10.3-26.9)	16.1 (10.2-22.0)	15.9 (8.4-23.4)	16.3 (8.2-24.3)
Asthma	281	1.4 (0.0-3.8)	2.2 (0.7-6.7)	0.7 (0.0-4.8)	1.9 (0.2-3.6)	2.3 (0.0-4.8)	1.5 (0.0-3.8)
Sensitisation	158	26.6 (20.2-34.1)	28.8 (19.7-39.8)	24.4 (16.0-35.3)	29.0 (22.2-35.7)	30.0 (21.2-38.8)	28.0 (18.8-37.1)
Cumulative incidence							
AD (question.)	274	28.8 (23.7-34.5)	27.3 (20.3-35.6)	30.3 (23.2-38.4)	32.7 (27.3-38.2)	32.9 (25.0-40.8)	32.6 (25.1-40.0)
AD (UKWP)	163	35.6 (28.4-43.3)	27.2 (18.5-38.0)	43.9 (33.5-54.9)	34.1 (27.8-40.4)	29.3 (21.2-37.4)	39.1 (30.2-48.0)
ARC	279	15.1 (11.3-19.8)	16.4 (11.0-23.7)	13.8 (9.0-20.5)	20.2 (15.3-25.2)	21.6 (14.6-28.6)	18.8 (12.0-25.7)
Asthma	281	6.0 (3.8-9.5)	6.6 (3.5-12.3)	5.5 (2.8-10.7)	6.8 (3.9-9.8)	6.4 (2.3-10.5)	7.3 (3.0-11.6)

AD: Atopic dermatitis; UKWP: UK Working Party diagnostic critera for AD; ARC: allergic rhinoconjunctivitis; [a]question.: questionnaire defined AD

Table s6: Summary statistics for comparison of imputed covariates and outcomes

	Observed data, % (95% CI)				Imputed estimates, % (95% CI)		
Variable	**N**	**Total population**	**Probiotic**	**Placebo**	**Total population**	**Probiotic (n=211)**	**Placebo (n=204)**
1 year symptoms							
Wheeze	278	16.9 (12.9-21.8)	12.7 (8.0-19.6)	20.8 (14.9-28.3)	18.9 (14.1-23.6)	15.1 (8.8-21.4)	22.8 (15.6-29.9)
Bronchitis	270	7.7 (5.1-11.7)	8.5 (4.7-14.7)	7.1 (3.9-12.9)	10.7 (6.6-14.8)	11.6 (5.8-17.4)	9.7 (4.4-15.0)
2 year outcomes							
Wheeze	279	30.5 (25.3-36.2)	29.9 (22.9-37.9)	31.1 (23.8-39.5)	31.8 (26.2-37.4)	31.5 (23.7-39.2)	32.1 (24.3-39.9)
Bronchitis	297	14.5 (10.9-19.0)	12.1 (7.7-18.4)	16.9 (11.6-23.9)	16.2 (11.7-20.6)	14.0 (8.1-19.8)	18.4 (12.0-24.9)
6 year outcomes							
Wheeze	274	19.7 (15.4-24.9)	17.4 (11.8-25.0)	21.8 (15.7-29.5)	20.7 (15.8-25.6)	17.4 (11.1-23.7)	24.1 (16.7-31.6)
Bronchitis	269	14.1 (10.4-18.9)	13.8 (8.8-21.0)	14.4 (9.4-21.4)	14.9 (10.8-19.1)	13.5 (7.7-19.3)	16.4 (10.1-22.6)
Pneumonia	279	11.8 (8.5-16.2)	9.0 (5.1-15.2)	14.5 (9.6-21.3)	12.7 (8.6-16.9)	10.2 (4.5-15.9)	15.4 (9.4-21.4)

Details of Pattern Mixture Methods (PMM) sensitivity analysis continued…

Table s7: Range of informative missingness odds ratio (IMOR) tested in sensitivity analysis

Disease	n_{miss}	Estimated proportion	Lower – upper end of ranges considered		
			Proportion of missing with disease	Corresponding total proportion with disease	Selected IMOR range
Current disease					
Asthma	134	1.6 (0.0-3.2)	0 – 20%	1.0 – 7.2%	0.00 – 5.00
Sensitisation	284	28.9 (22.1-35.8)	10 – 50%	16.9-44.3%	0.20 – 2.20
Cumulative incidence					
AD (UKWP)	252	31.3 (25.2-37.4)	5 – 50%	16.9 – 50.4%	0.09 – 2.84
ARC	136	18.5 (13.7-23.4)	5 – 40%	11.6 – 23.1%	0.25 – 3.75

AD: atopic dermatitis; ARC: allergic rhinoconjunctivitis

Results and discussion:

Large deviations from the MAR assumption in a single treatment arm are required before probiotic supplementation would have had a statistically significant effect on the cumulative incidence of ARC, 12 month prevalence of asthma or current atopic sensitisation (Figure s1). Small violations of the MAR assumption in the probiotic or placebo arm, but not both, would have resulted in a reduction or increase of the effect on maternal probiotic supplementation on the cumulative incidence of AD diagnosed using the UKWP diagnostic criteria. That is to say, if children with AD were slightly more likely to have been lost to follow-up in the probiotic group, then the observed OR for the cumulative incidence of AD in the probiotic group would have been closer to 1. Conversely, if the children with AD were slightly more likely to be lost to follow-up in the placebo group, then the beneficial effect of probiotics would have been even greater. Erring on the side of caution we observe that, had the odds ratio (OR) between UKWP-defined cumulative AD and missingness been more than ~1.12 in the probiotic group or less than ~0.88 in the placebo group, the observed association between probiotic supplementation and cumulative incidence of AD would not have been statistically significant. Although it is not expected that there is a differential association between AD and the risk of drop-out in each treatment arm, we can conclude that relatively small differences would affect the results and thus the magnitude of the effect of probiotics and size of the type 1 error must be interpreted with caution.

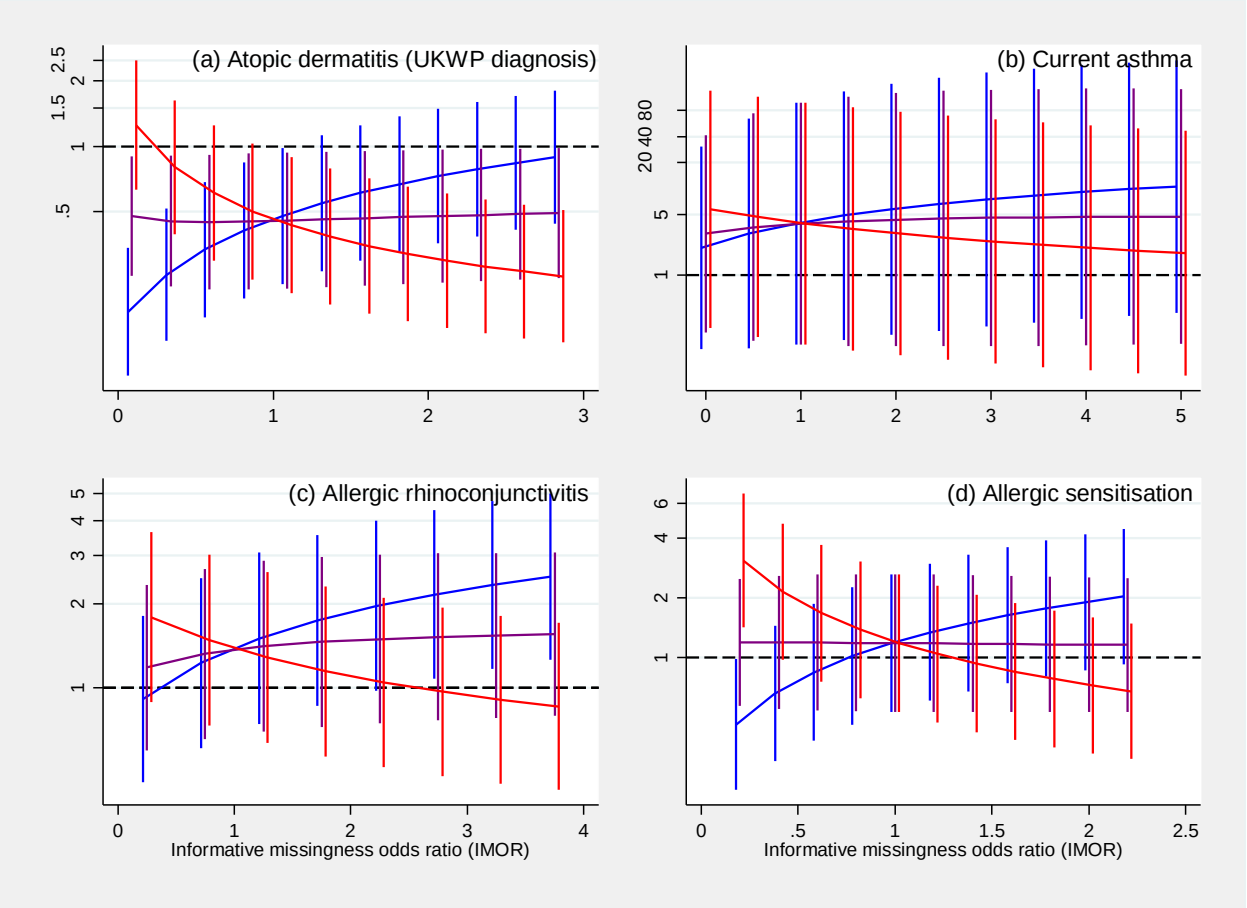

Figure s1: Pattern mixture model sensitivity analysis for estimate of probiotic effect on the cumulative incidence of AD (panel (a)), 12 month prevalence of asthma (b), cumulative incidence of ARC (c) and current allergic sensitisation (d) at 6 years. Each graph depicts the impact of deviations from MAR in the probiotic group only (blue line), placebo group only (red line) and both groups (purple line).

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