

ORIGINAL ARTICLE

EPIDEMIOLOGY AND GENETICS

Vitamin A supplementation and BCG vaccination at birth may affect atopy in childhood: long-term follow-up of a randomized controlled trial

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Abstract

Background: Recent evidence suggests that immunogenic interventions such as vaccines and micronutrients may affect atopic sensitization and atopic disease. We aimed to determine whether neonatal BCG vaccination, vitamin A supplementation and other vaccinations affect atopy in childhood.

Methods: In Guinea-Bissau, low-birthweight infants were randomized to early (intervention) or delayed (usual policy) BCG. A subgroup was also randomly assigned vitamin A supplementation or placebo in a two-by-two factorial design. Participants were followed up at age 3–9 years. The main outcome was atopy defined as skin prick test reaction ≥ 3 mm. Secondary outcomes were symptoms of eczema, asthma and food allergy.

Results: Two hundred eighty-one children had valid skin prick tests performed, and 14% (39/281) were atopic. There was no significant difference in atopy between the early and delayed BCG groups (OR, 0.71; 95% CI, 0.34–1.47). Atopy was significantly reduced in children who had responded to BCG with a scar (OR, 0.42; 0.19–0.94). Vitamin A supplementation was associated with increased atopy (OR, 2.88; 1.26–6.58), especially in those who received simultaneous BCG (5.99; 1.99–18.1, $P = 0.09$ for interaction between vitamin A supplementation and BCG). Early vs delayed BCG was not associated with symptoms of atopic disease, but vitamin A supplementation increased odds of wheeze within the past 12 months (OR, 2.45; 1.20–4.96).

Conclusions: There were no statistically significant effects of early vs delayed BCG on atopy or symptoms of atopic disease. Having a BCG scar was associated with reduced atopy, whereas neonatal vitamin A supplementation was associated with increased atopy.

Study Registration: Clinicaltrials.gov NCT 01420705.

The prevalence of allergic disease in childhood is increasing globally, both in high-income countries and in poor countries (1). The hypothesis that microbial exposure and infections protect against atopic disease offers an explanation for this change (2). Live routine vaccinations evoke a similar immune response to wild-type infections and have also been associated with protection from atopic disease, particularly Bacille Calmette-Guérin (BCG) (3–5). In an observational study, we found that BCG was highly protective against atopic sensitiza-

tion with the greatest protection when the vaccine was given in the first week of life (5). Despite potential for considerable protection, only one small randomized trial of BCG for protection against atopic disease has been performed, finding a trend towards less eczema and significantly less use of eczema medication at 18 months in infants BCG vaccinated at 6 weeks of age, but no difference in other outcomes (6).

The Bandim Health Project (BHP) conducted a trial in Guinea-Bissau amongst low-birthweight infants who were

randomly allocated to BCG vaccination at birth or later as is local policy (7, 8). A subgroup of children was furthermore randomized to vitamin A or placebo at birth. The primary outcome was infant mortality. This cohort provided an opportunity to study the effect of timing of BCG vaccination on atopic sensitization later in childhood. As observed previously (5), our *a priori* hypothesis was that early BCG would be protective against atopy, whereas early diphtheria–tetanus–pertussis vaccination (DTP) would increase atopy (9). We furthermore studied the effect of vitamin A supplementation.

Methods

Setting and population

In 2002, the BHP commenced a randomized trial of early BCG vaccination at the three local health centres in the BHP study area and at the National Hospital, as described elsewhere (7, 8). Inclusion criteria were the same throughout the trial; however, children born before November 2004 at the National Hospital were excluded from the cohort due to randomization errors as previously described (7, 8). At enrolment, demographic and anthropometric data were collected, including maternal mid-upper arm circumference (MUAC). Our sample for this follow-up study was restricted to 808 children coming from the BHP study area.

One hundred six of the 808 children had died before the most recent health and demographic surveillance system visit. Most deaths were due to respiratory infections, sepsis, gastrointestinal infections and fever/malaria (7, 8, 10). Of the 702 remaining children, 317 (45%) were still living in the study area, of whom 289 (91%) were included in the follow-up study (Fig. 1). 152 (53%) children had also been enrolled in the vitamin A supplementation (VAS) trial.

Interventions with BCG and vitamin A

In the randomized trial, low-birthweight children (<2.5 kg) were randomly allocated to early BCG (0.05 ml) ($1-4 \times 10^5$ colony-forming units) of Statens Serum Institut Danish strain BCG by intradermal injection) or control group at a median age of 2 days (Table 1) (7, 10). Those allocated to control group were instructed to return to a local health centre for BCG when the child was >2.5 kg or was due to receive DTP at 6 weeks of age as is usual policy for low-birthweight infants. 51% (410/808) of the cohort were also randomly allocated to 25 000 IU oral vitamin A or placebo at the National Hospital in a two-by-two factorial design between May 2005 and January 2008 to document the effect of VAS on all-cause infant mortality (10).

Other exposures: measles vaccine and DTP vaccine

At the age of 4.5 months, 35% of the cohort (281/808) took part in a separate trial in which two-thirds of the children were randomized to an extra doses of measles vaccine (11). At the time of the study, 0.5 ml of DTP (containing

whole-cell pertussis) delivered via the intramuscular route was routinely recommended at six, 10 and 14 weeks of age. We evaluated the effect of routinely delivered DTP on atopy using a distinction between early (≤ 8 weeks of age) and late (> 8 weeks of age) first dose of DTP (12).

Follow-up for atopy

Children were visited at home between October and December 2011 and parental informed consent obtained. Children were excluded from skin prick test if they had a history suggestive of anaphylaxis ($n = 2$) or were currently using antihistamine medication ($n = 2$). Children had weight, height and MUAC measured. Bacille Calmette–Guérin scar was examined, and vaccination details were recorded by the assistant who was not performing the skin prick test.

Skin prick test was performed using three aero-allergens (*Dermatophagoides pteronyssinus* and *D. farinae* mix, *Blomia tropicalis* and *Blattella germanica*), three food allergens (cow's milk, egg white and peanut) and positive and negative controls (ALK-Abelló, Madrid, Spain). House dust mites, cockroach and *Blomia tropicalis* are the most common allergens to which children in Guinea-Bissau are sensitized (13). All skin prick tests were performed by one researcher (NK) who was blinded to vaccination status. Atopy was defined as an average wheal diameter ≥ 3 mm (after subtracting the negative control) for any allergen. Children with a positive control < 2 mm ($n = 4$) were excluded from atopy analysis.

Symptoms of eczema and asthma were assessed using questions from the ISAAC questionnaire (1), translated into Portuguese and interpreted into Creole. Skin reactions, facial swelling, respiratory symptoms, vomiting or syncope within 1 h of ingestion of food was considered likely to indicate a reaction to food. The study protocol was approved by the National Ethical Committee in Guinea-Bissau and by the Human Research Ethics Committee, Royal Children's Hospital, Melbourne, Australia.

Statistical analysis

Demographic data and anthropometry were compared using chi-square and Kruskal–Wallis tests. Effect of interventions and other factors were analysed using logistic regression, adjusting for potential interdependency of outcomes within twin pairs using robust standard errors.

In the primary analysis, we compared the early vs delayed BCG groups. Because there was evidence of interaction between the two concurrent interventions, BCG and VAS, we adjusted estimates of the effect of BCG for VAS and vice versa and present stratified results. We adjusted for age (3–4, 5, 6, 7–9 years) and sex in all analyses as there were differences in age between participants and nonparticipants in the VAS trial, and vaccinations have sex-differential nonspecific effects (14).

In the secondary analysis of timing of BCG, DTP and number of doses of measles vaccine (Table 3), odds ratios (OR) were adjusted for VAS and each of the vaccinations. Associations between demographic and environmental factors and atopy presented in Supplementary Table S3 were

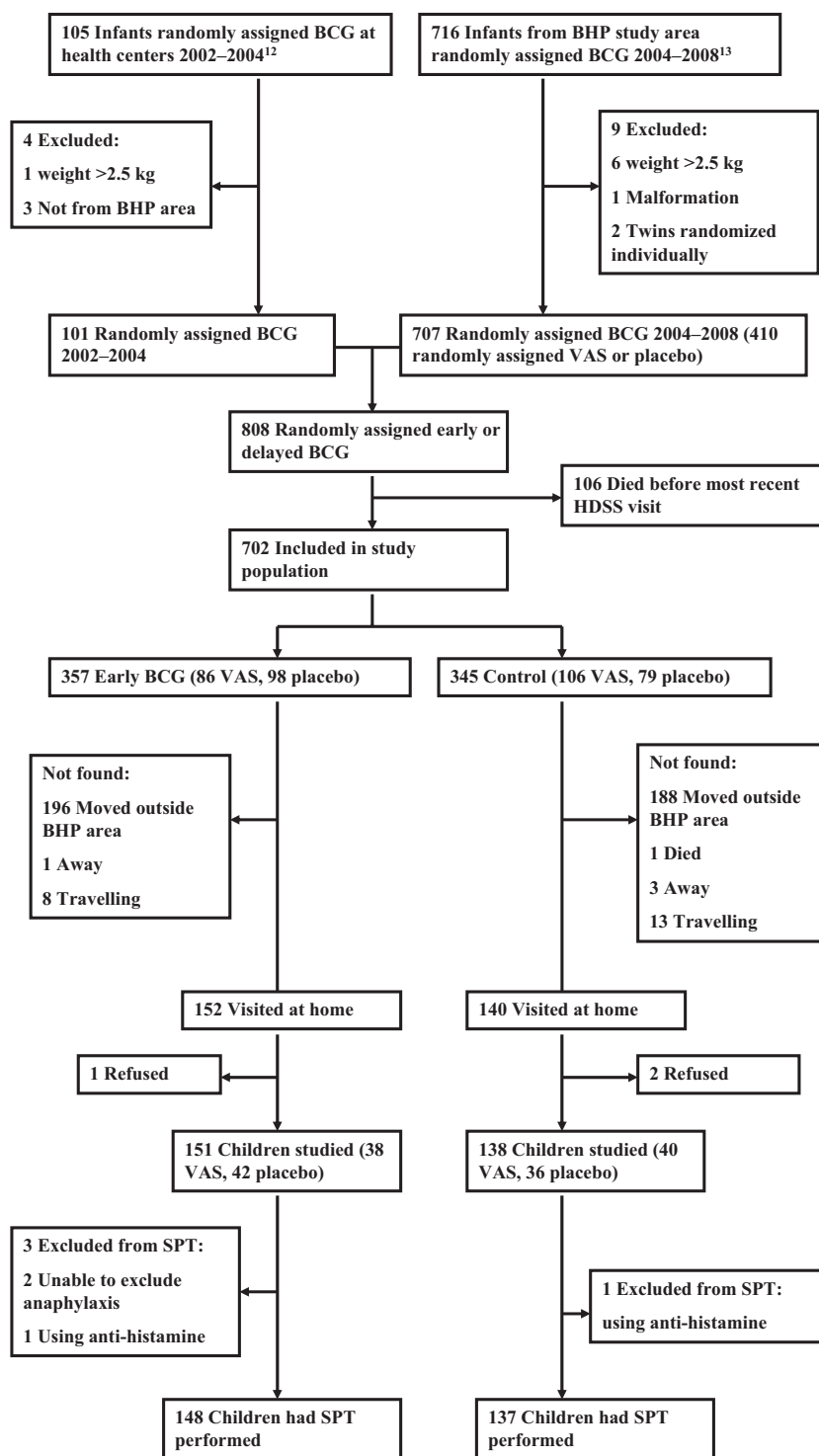


Figure 1 Study profile. BHP, Bandim Health Project; VAS, vitamin A supplementation.

adjusted for all factors in the table except anthropometry at birth due to missing observations. Missing information was excluded only from relevant analyses. *P* values less than 0.05 were considered statistically significant. Various comparisons

are made in the presented analyses; no formal mathematical correction was made for multiple comparisons. The results should be interpreted taking this into account. Statistical analyses were performed using Stata 11.

Results

Study participants

Demography and environmental characteristics are shown in Table 1 for the early BCG and control groups. A greater percentage of control children were DTP- and polio-vaccinated at 12 months than the early BCG group. Median age of BCG vaccination was 2 days in the early BCG group and 47 days in the control group (Table 1). Median age of VAS or placebo administration was 1 day (Table S2). Demographic differences

between children assigned to different protocols are outlined in Tables S1 and S2. When adjusted for age, there were no differences in anthropometry at enrolment or at follow-up between those who did and did not receive VAS ($P > 0.10$ for all comparisons).

Prevalence of atopy

Of the 281 children for whom skin prick test results were analysed, 39 (14%) were atopic. Positive reactions were more

Table 1 Demography and anthropometry for early BCG and control groups

	Early BCG group ($n = 151$)	Control group ($n = 138$)	P value or OR (95% CI)
At enrolment of BCG trial			
Maternal MUAC (mm)	250 (208–285) $n = 138$	243 (212–280) $n = 116$	0.23
Age (days)	2 (1–13) $n = 151$	3 (1–12) $n = 138$	0.77
Girls	83/151 (55%)	81/138 (59%)	0.52
Twins	30/151 (20%)	27/138 (20%)	0.95
Weight (g)	2.21 (1.73–2.40) $n = 151$	2.18 (1.66–2.43) $n = 138$	0.35
Length (cm)	46.0 (43.0–48.0) $n = 151$	45.5 (43.0–47.5) $n = 137$	0.15
MUAC (mm)	82 (70–88) $n = 151$	82 (70–88) $n = 136$	0.30
At 12-months visit			
Weight (kg)	8.08 (6.92–9.54) $n = 133$	8.20 (6.72–9.56) $n = 127$	0.67
Height (cm)	71.0 (67.0–74.0) $n = 126$	70.7 (66.7–75.0) $n = 118$	0.92
DTP3 vaccinated	118/132 (89%)	117/122 (96%)	0.05
OPV3 vaccinated	117/132 (89%)	119/122 (98%)	0.01
BCG vaccinated in control group		127/138 (92%)	
At follow-up for atopy			
Age (years)	5.6 (4.1–7.5) $n = 151$	5.6 (4.2–7.2) $n = 138$	0.61
Weight (kg)	16.2 (13.5–21.2) $n = 151$	15.9 (13.0–20.0) $n = 137$	0.18
Height (cm)	108.5 (97.5–122.0) $n = 151$	107.5 (96.0–119.0) $n = 138$	0.32
MUAC (mm)	160 (148–176) $n = 151$	158 (146–174) $n = 136$	0.18
Used medication in past 3 days	21/150 (13%)	17/138 (12%)	0.67
History of hospitalization	56/151 (37%)	50/137 (36%)	0.92
Has ≥ 1 older sibling	105/151 (70%)	88/138 (64%)	0.30
Animals at house	61/151 (40%)	42/138 (30%)	0.08
Breastfeeding >18 months	120/151 (79%)	104/138 (75%)	0.40
Mother answered survey	131/151 (87%)	110/138 (80%)	0.11
BCG scar present	131/150 (87%)	110/134 (82%)	0.22
Scar >5 mm in children who have a scar	34/131 (26%)	27/110 (25%)	0.80
Enrolled in VAS trial	80/151 (53%)	76/138 (55%)	0.72
Randomized to VAS	38/80 (47%)	40/76 (53%)	0.52
Age of BCG vaccination (days)	2 (1–12) $n = 151$	47 (21–71) $n = 133$	<0.001
Atopy			
Skin prick reaction ≥ 3 mm	17/145 (12%)	22/136 (16%)	0.71 (0.34–1.47)
Symptoms of atopic disease			
Eczematous rash	2/149 (1%)	5/137 (4%)	0.36 (0.07–2.05)
Wheeze ever	43/150 (29%)	33/138 (24%)	1.31 (0.76–2.28)
Wheeze in past 12 months	24/149 (16%)	20/138 (14%)	1.18 (0.60–2.36)
Exercise-induced wheeze	6/151 (4%)	5/138 (4%)	1.08 (0.32–3.64)
Dry cough after exercise	30/150 (20%)	32/138 (23%)	0.83 (0.46–1.50)
Nocturnal cough	14/150 (9%)	7/138 (5%)	1.92 (0.68–5.37)
Reaction to food	14/149 (9%)	11/137 (8%)	1.21 (0.54–2.72)

Data are number (%) or median (10th to 90th centiles).

Some data are missing as indicated by the denominator. OR for atopy calculated by logistic regression adjusted for age, sex and VAS. OR for symptoms of atopic disease calculated by logistic regression with adjustment for VAS only due to low numbers in some groups.

OR, odds ratio; MUAC, mid-upper arm circumference; VAS, vitamin A supplementation.

often related to aero-allergens than food allergens (Fig. S1). Of the seven children who had reactions to food allergens, three were sensitized to cow's milk, two to egg white and two to peanut.

BCG vaccination

Virtually, all children were BCG vaccinated (Table S1). Atopy was insignificantly reduced in the early BCG group compared with the control group (OR 0.71; 0.34–1.47, Table 2). However, the effect of BCG tended to differ between children who had received VAS and those who had not. The OR for early BCG vs controls was 1.50 (0.46–4.92) in children who received VAS and was 0.40 (0.15–1.06) in children who did not receive VAS ($P = 0.09$ for interaction between BCG and VAS, Table 2). Adjusting for polio and DTP vaccinations at the 12-months visit made little difference to these estimates (data not shown). The OR of atopy for early BCG vs controls was 0.40 (0.13–1.25) for boys and 1.10 (0.41–2.92) for girls.

We also examined the effect of age of BCG vaccination (Table 3). In children who did not receive VAS, the odds of atopy increased with greater delay until BCG vaccination (P for trend 0.03), with almost sixfold increase in atopy in children who received BCG after 8 weeks (Table 3).

Eighty-five percent of children responded to BCG with a scar (Table 1). Amongst those with a scar, 12% (28/233) were atopic compared with 26% (11/43) amongst those without a scar (OR, 0.42; 95% CI, 0.19–0.94). Maternal MUAC was positively correlated with having a BCG scar ($P = 0.03$). Adjustment for maternal MUAC in the 242 children who

had maternal MUAC data available did not change the association between BCG scar and atopy (OR, 0.40; 0.17–0.96). There were no associations between other background or interventional factors and BCG scar.

Neonatal vitamin A supplementation

Children who received VAS had significantly increased atopy compared with children who did not receive VAS (OR, 2.88; 1.26–6.58, Table 2). The OR was 3.39 (1.19–9.65) in girls and 2.36 (0.72–7.78) in boys. The increase in atopy was most pronounced in children who received early BCG, whereas there was no significant effect in the control group (Table 2). The effect size was similar when the analysis was restricted to children enrolled in the VAS trial (Table 2), and a similar pattern was seen for skin prick reactions ≥ 2 mm (Table S4). As maternal MUAC and the child's length at birth were associated with atopy and differed according to enrolment in the VAS trial, we adjusted the odds ratio for these factors: the effect of VAS remained (OR, 3.41; 1.32–8.79). Similarly, the effect remained after adjustment for weight, height and MUAC at follow-up (OR 3.08; 1.34–7.11). The negative effect of VAS on atopy was greatest in children in the early BCG group and those who received DTP before 8 weeks of age (Table S5).

Other exposures: measles vaccine and DTP vaccine

The 86 children who received a second dose of measles vaccine in the measles vaccination trial (11) had reduced atopy compared with all children not allocated the extra measles

Table 2 Two-by-two analyses of the effect of early BCG vaccination and vitamin A supplementation on atopy

	All children <i>n</i> = 281	Early BCG group <i>n</i> = 145	Control group <i>n</i> = 136	Odds ratio early BCG vs control (95% CI)	<i>P</i> value
Atopy in all children* (<i>n</i> = 281)	39/281 (14%)	17/145 (12%)	22/136 (16%)	0.71 (0.34–1.47)	0.35
Stratified by VAS					
VAS	18/76 (24%)	10/37 (27%)	8/39 (21%)	1.50 (0.46–4.92)	0.50
No VAS	21/205 (10%)	7/108 (6%)	14/97 (14%)	0.40 (0.15–1.06)	0.07
Odds ratio VAS vs no VAS (95% CI)	2.88 (1.26–6.58)	5.99 (1.99–18.1)	1.59 (0.50–5.02)		
<i>P</i> value	0.01	0.001	0.43		
			Interaction between early BCG and VAS		0.09
Atopy restricted to children in vitamin A trial† (<i>n</i> = 152)	26/152 (17%)	12/77 (16%)	14/75 (19%)	0.82 (0.32–2.10)	0.68
Stratified by VAS					
VAS	18/76 (24%)	10/37 (27%)	8/39 (21%)	1.46 (0.46–4.63)	0.52
Placebo	8/76 (11%)	2/40 (5%)	6/36 (17%)	0.25 (0.04–1.46)	0.12
Odds ratio					
VAS vs placebo (95% CI)	2.70 (0.98–7.43)	7.57 (1.48–38.7)	1.28 (0.34–4.89)		
<i>P</i> value	0.05	0.02	0.72		
			Interaction between early BCG and VAS		0.10

Data are number (%).

*VAS vs placebo and those not enrolled in vitamin A trial.

†VAS vs placebo. Odds ratios are adjusted for age and sex.

VAS, vitamin A supplementation.

Table 3 Routine vaccinations and prevalence of atopy, stratified by vitamin A supplementation

	All children (<i>n</i> = 281)		Vitamin A supplementation (<i>n</i> = 76)		No vitamin A supplementation (<i>n</i> = 205)	
	Atopy	Odds ratio (95% CI)	Atopy	Odds ratio (95% CI)	Atopy	Odds ratio (95% CI)
BCG vaccination age						
<1 week	11/108 (10%)	1.00 (ref)	6/30 (20%)	1.00 (ref)	5/78 (6%)	1.00 (ref)
1–4 weeks	10/55 (18%)	2.14 (0.79–5.82)	6/17 (35%)	1.84 (0.38–8.95)	4/38 (11%)	2.45 (0.62–9.73)
5–8 weeks	13/85 (15%)	1.55 (0.60–4.01)	5/21 (24%)	0.87 (0.18–4.27)	8/64 (12%)	2.34 (0.71–7.77)
>8 weeks or no BCG	5/33 (15%)	3.04 (0.80–11.5)	1/8 (12%)	0.93 (0.09–9.90)	4/25 (16%)	5.84 (1.27–26.9)
	<i>P</i> for trend	0.13	<i>P</i> for trend	0.84	<i>P</i> for trend	0.03
			<i>P</i> for interaction between BCG timing and VAS			0.13
DTP vaccination 1st dose						
>8 weeks of age	7/78 (9%)	1.00 (ref)	2/17 (12%)	1.00 (ref)	5/61 (8%)	1.00 (ref)
≤8 weeks of age	32/203 (16%)	2.76 (0.91–8.41)	16/59 (27%)	4.71 (0.61–36.6)	16/144 (11%)	2.10 (0.67–6.61)
			<i>P</i> for interaction between DTP timing and VAS			0.45
Measles vaccine						
No extra dose of MV	30/195 (15%)	1.00 (ref)	13/52 (25%)	1.00 (ref)	17/143 (12%)	1.00 (ref)
Extra dose of MV	9/86 (10%)	0.38 (0.15–0.96)	5/24 (21%)	0.45 (0.12–1.71)	4/62 (6%)	0.33 (0.09–1.18)
			<i>P</i> for interaction between extra dose of MV and VAS			0.73

Data are number (%). Odds ratios are adjusted for age, sex, vitamin A supplementation and other vaccinations in the table. DTP, diphtheria–tetanus–pertussis vaccination; MV, measles vaccine; VAS, vitamin A supplementation.

vaccine (OR, 0.38; 0.15–0.96, Table 3). The effect size was similar when restricting the analysis to the 133 children enrolled in the measles vaccine trial (OR, 0.45; 0.16–1.27).

As shown elsewhere, it was the healthiest children who were DTP vaccinated before 8 weeks of age (12). Nonetheless, receiving DTP before 8 weeks of age tended to be associated with increased atopy compared with receiving DTP later (OR, 2.76; 0.91–8.41, Table 3).

Symptoms of atopic disease

The most common symptoms reported were respiratory symptoms (Table 1). The odds of wheeze within the past 12 months were increased in children who received VAS compared with those who did not receive VAS (OR, 2.45; 1.20–4.96, Table S6). The effect size was similar when restricted to children enrolled in the VAS trial (OR, 2.59; 0.95–7.04). There was no other significant association with allergic outcomes.

Discussion

Early BCG had no significant overall effect on the development of atopy compared with delayed BCG. However, those who had developed a BCG scar had half the odds of atopy compared to those without a scar. VAS was associated with almost threefold increase in atopy. There was evidence of interaction between VAS and BCG vaccination, the two randomized interventions delivered simultaneously. Children tended to have reduced atopy if they received BCG at birth *vs* delayed BCG only if they did not receive VAS. Conversely, VAS increased atopy almost sixfold if children received early BCG, but only by 1.6-fold in those who had delayed BCG.

In the secondary analysis of vaccination timing, those who had BCG delayed by more than 8 weeks had almost sixfold increase in atopy (if they did not receive VAS). Also, a second dose of measles vaccine halved the odds of atopy, whereas there was a trend towards increased atopy (2.6-fold) associated with early DTP, as hypothesized *a priori*.

These associations between vaccinations and atopy have precedents. A small randomized trial found a trend towards less eczema and significantly less use of eczema medication associated with BCG (6). Bacille Calmette–Guérin has been linked to less atopy, asthma and hay fever in some observational studies but not in others (3–5), as has presence of a BCG scar (3). It is unclear whether the association between BCG scar and protection from atopy in the present study was due to difference in the immune response to the BCG vaccination or in fact related to the correct administration of the intradermal vaccination. However, the finding that timing of BCG in infancy affected expression of atopy indicates that there is an effect that is independent of underlying biological differences in the children, and the effect is consistent with our findings in Guinea-Bissau 16 year ago (5).

Recent epidemiological studies of pertussis-containing vaccines and atopic disease comparing vaccinated with unvaccinated children have had mixed results (15–18). A 2007 meta-analysis of whole-cell pertussis vaccination and asthma found evidence of increased risk of asthma after pertussis vaccination amongst the five highest quality studies, although no significant effect was found when including all seven eligible studies (19). Some of these studies used any vaccination as the exposure, while some used completion of the primary series of three doses, and timing was not taken into consideration. However, a number of observational studies have reported an association between DTP timing and asthma or

hay fever. Atopic disease was associated with early DTP vaccination in studies from the UK and Canada (9, 20), whereas data from a UK cohort reported no association between timing of DTP and asthma or eczema (21). All these studies examined DTP with the whole-cell pertussis component, and no studies have explored timing of the acellular vaccine. Importantly, no previous studies analysed timing of BCG and DTP in a multivariate analysis. Our previous study in Guinea-Bissau found that DTP appeared to be protective against atopy until the effect was adjusted for BCG, revealing a significant harmful effect of DTP (5). Our findings indicate that timing of both vaccines is related to atopy with opposing effects, and previous studies of vaccination timing may therefore have been confounded by delay in both vaccines for common reasons.

There is only one small study on VAS and atopy by our group which found no strong effect of VAS with measles vaccine at age 6 and 9 months on atopy later in childhood (22). A recent systematic review found no interventional studies of VAS, and of all the studies examining dietary intake of vitamin A, none found a protective or provocative effect on atopy or symptoms of atopic disease (23). While some studies in the review found vitamin A deficiency to be associated with increased risk of atopic disease (23), there is laboratory evidence that vitamin A deficiency may be protective against asthma and that VAS is associated with increased airway hyper-responsiveness (24). It is noteworthy that neonatal VAS has no impact on serum vitamin A levels beyond 6 weeks of age (25). Hence, the effect we found of neonatal VAS on atopy in childhood is probably unrelated to current vitamin A status. This study is not the first to find long-lasting effects of neonatal VAS on child health. We previously reported that neonatal VAS may have interacted negatively with both DTP vaccine given around 6 weeks of age (26) and with early measles vaccine at 4.5 months of age (11). Also, neonatal VAS primed the immune response to a subsequent dose of VAS in girls at age 12 months (27). Binding of retinoids to the RAR/RXR has been shown to initiate a cascade of changes in chromatin structures, which can initiate stable epigenetic changes (28). Such epigenetic dysregulation of B lymphocytes has been identified as a key factor in subjects with allergy to house dust mite compared with non-allergic subjects (29). We speculate that neonatal VAS induces important epigenetic changes with fundamental consequences for the developing immune system and its response to other interventions as well as to allergens.

Furthermore, there is currently intense interest in the role of vitamin D in allergic disease (30). Recent epidemiological evidence suggests that vitamin D deficiency may be a risk factor for eczema (31) and asthma (30). Current vitamin D deficiency was also a risk factor for food allergy at age one in Australian children (32), but maternal and newborn vitamin D deficiency was protective for food allergy at age two in a lower prevalence German cohort (33).

The associations between routine interventions and childhood atopy found in this study show a remarkably similar pattern to those found between the same interventions and all-cause mortality in high-mortality countries, including

BCG (7, 8, 14), VAS (10, 26, 34), measles vaccination (11) and DTP (12). Such similarities suggest that there may be common biological mechanism, potentially involving the innate immune system (35). Recent evidence of epigenetic reprogramming of monocytes by BCG has offered a viable mechanism by which BCG provides heterologous immunity to unrelated pathogens (36). Immunological studies on potential mechanisms by which BCG may protect against atopic disease are warranted.

This study had a number of weaknesses. For logistical reasons, we were only able to follow children still living within the study area which was 37% of the original cohort due to deaths and movements. However, we know of no reason that this restriction to children from the study area could bias our findings. More children died in the control group *vs* the early BCG group before one year of age (8). If frail children with high risk of atopy died in the control group but survived in the early BCG group, it may have diluted a protective effect of BCG vaccination.

Children born 2002–2004 were not included in the VAS trial, and therefore, there were differences in age and anthropometry between those who did and did not receive VAS. We demonstrated that these differences are unlikely to have biased our VAS findings by showing that the effect size was almost identical when restricted to the randomized children. Although age itself was not associated with atopy, we adjusted all analyses for age. Furthermore, the harmful effect of VAS remained after adjustment for maternal MUAC and infant length, the two factors that differed between VAS trial participants and correlated with atopy. Finally, there were weaknesses in the outcomes. Skin prick tests were performed by one trained researcher, were not duplicated and there was no ongoing control of proficiency. In the results of the survey, although prevalence of 'current wheeze' was similar in Guinea-Bissau to neighbouring populations (37), it correlated poorly with atopic sensitization and was largely unaffected by environmental factors, indicating that the survey has poor specificity for atopic disease. This may have been due to poor local understanding of atopic disease and difficulties in both translation and interpretation of the survey. Therefore, the results should not be directly compared with other similar data sets.

In conclusion, there was no statistically significant effect of early *vs* delayed BCG on atopy or symptoms of atopic disease. However, having a BCG scar was associated with reduced atopy and in children who did not receive VAS, increasing delay in BCG was associated with increasing risk of atopy. These results support our previous suggestion that BCG vaccination at birth would be beneficial for low-birth-weight infants in Guinea-Bissau (7). Given the potential for considerable protection against atopic disease, large randomized trials of neonatal BCG for the prevention of atopic disease in unvaccinated populations are needed.

There is strong support to have neonatal VAS included in WHO policy for countries at high risk of vitamin A deficiency (38–40), and three large randomized trials to inform global policy are underway in Ghana, India and Tanzania, testing the effect of neonatal VAS on mortality (41). The

potential atopy-increasing effect of neonatal VAS documented in the present study is therefore worrying.

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Author contributions

All authors contributed to study planning and the final draft of the manuscript. NK performed the fieldwork, analysed the data, interpreted results and wrote the first draft of the manuscript. CSB was the PI of the vitamin A trial and provided help with the data analysis and interpreted the results. SBS coordinated logistics and supervised fieldwork. AR and KJJ coordinated logistics. HR provided help with the data analysis and interpreted results. KA interpreted the results. PA was the PI of the BCG trial, supervised the project, provided help with the data analysis and interpreted the results. All authors had full access to the data and analyses.

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Conflict of interest

Several authors are affiliated with the Statens Serum Institut (SSI) in Copenhagen, which administers their grants. SSI is a producer of BCG. However, SSI did not fund the vaccines, the study or the researchers. The authors declare that they have no other conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Allergens eliciting atopic skin prick reaction. Food allergens are egg white, cow's milk and peanut.

Table S1. Demography and anthropometry according to participation in follow-up study.

Table S2. Demography and anthropometry according to vitamin A trial group.

Table S3. Anthropometry at birth, demography, and environmental factors and risk of atopy.

Table S4. Two-by-two analyses of the effect of early BCG vaccination and vitamin A supplementation on skin prick reaction ≥ 2 mm.

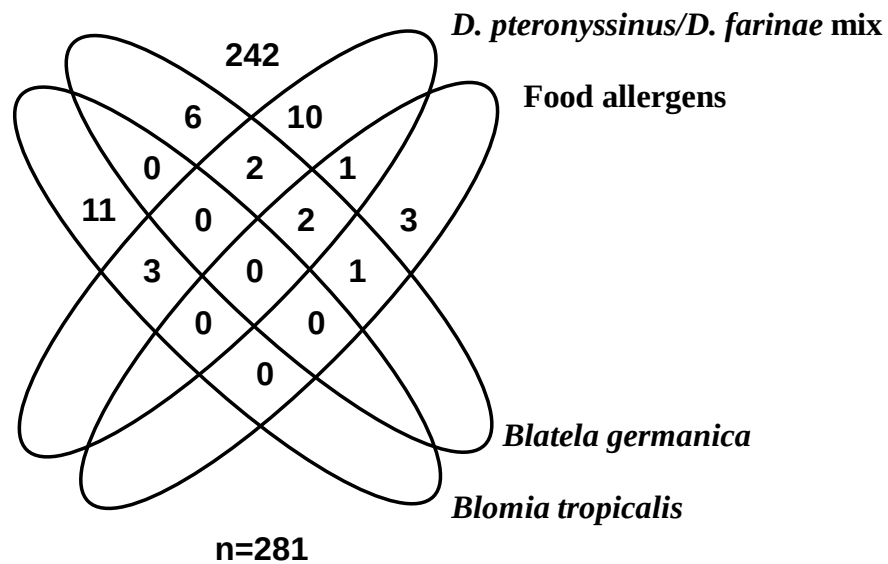
Table S5. Effect of vitamin A supplementation on atopy stratified by BCG randomization group and early or late DTP.

Table S6. Effect of vitamin A supplementation on symptoms of atopic disease.

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Supplementary Figure 1. Allergens eliciting atopic skin prick reactions. Food allergens are egg white, cow's milk and peanut

Supplementary table 1: Demography and anthropometry according to participation in follow-up study

	Population enrolled in BCG trial from BHP study area (n=808)	Population surviving to 12 months but not included in follow-up study (n=413)	Children included in follow-up study (n=289)	p value (not included vs. included children)
At enrolment of BCG trial				
Maternal MUAC (mm)	241 (208–280) n=708	238 (206–274) n=366	244 (208–280) n=254	0·05
Age (days)	2 (1–13) n=808	2 (1–13) n=413	2 (1–13) n=289	0·71
Girls	471/808 (58%)	251/413 (61%)	164/289 (57%)	0·28
Twin or triplet	165/808 (20%)	87/413 (21%)	57/289 (20%)	0·66
Weight (kg)	2·19 (1·66–2·42) n=807	2·20 (1·70–2·42) n=413	2·20 (1·70–2·42) n=289	0·44
Length (cm)	45·5 (42·5–48·0) n=805	46·0 (43·0–48·0) n=410	45·7 (42·7–47·5) n=288	0·34
MUAC (mm)	82 (70–88) n=804	82 (70–88) n=412	82 (70–88) n=288	0·76
Born at national hospital	503/808 (62%)	253/413 (61%)	191/289 (66%)	0·19
Enrolled in VAS trial	410/808 (51%)	208/413 (50%)	156/289 (54%)	0·34
Randomized to VAS	214/410 (52%)	112/208 (54%)	78/156 (50%)	0·47
At 12 month visit				
Weight (kg)		8·10 (6·84–9·62) n=252	8·14 (6·74–9·55) n=260	0·87
Height (cm)		71·0 (67·0–74·0) n=228	71·0 (67·0–74·5) n=244	0·99
DTP3 vaccinated		203/239 (85%)	235/254 (93%)	0·01
OPV3 vaccinated		204/239 (85%)	236/254 (93%)	0·009
BCG vaccinated in control group		108/116 (93%)	117/122 (96%)	0·35

Data are number (%) or median (10th – 90th centiles). MUAC=mid-upper arm circumference. VAS=vitamin A supplementation. DTP3=3rd dose of DTP vaccination, expected at 14 weeks according to national policy. OPV3=3rd dose of oral polio vaccine, expected at 14 weeks.

Supplementary table 2: Demography and anthropometry according to vitamin A trial group

	Vitamin A (n=78)	Placebo (n=78)	Not included in VAS trial (n=133)	p value
At enrolment of BCG trial				
Maternal MUAC (mm)	238 (202–280) n=77	244 (214–280) n=78	250 (220–285) n=99	0·005
Age (days)	1 (1–9) n=78	2 (1–12) n=78	4 (1–13) n=133	<0·001
Twin or triplet	22/78 (28%)	20/78 (26%)	15/133 (11%)	0·004
Weight (kg)	2·16 (1·70–2·35) n=78	2·14 (1·54–2·40) n=78	2·22 (1·82–2·45)	0·009
Length (cm)	45·5 (42·0–47·5) n=77	45·0 (41·5–48) n=78	46·0 (43·5–48·0) n=133	0·01
MUAC (mm)	82 (70–88) n=78	76 (64–88) n=78	82 (70–90)	<0·001
Girls	43/78 (55%)	44/78 (56%)	77/133 (58%)	0·92
Born at national hospital	78/78 (100%)	78/78 (100%)	35/133 (%)	<0·001
At 12 month visit				
Weight (kg)	8·08 (6·60–9·28) n=69	8·25 (6·76–9·56) n=70	8·02 (6·76–9·76) n=121	0·63
Height (cm)	70·5 (65·5–75·0) n=67	71·0 (67·2–74·5) n=65	71·0 (67·0–74·5)	0·53
DTP3 vaccinated	61/68 (90%)	67/70 (96%)	107/116 (92%)	0·40
OPV3 vaccinated	62/68 (91%)	68/70 (97%)	106/116 (91%)	0·27
BCG vaccinated in control group	31/34 (91%)	31/32 (97%)	55/56 (98%)	0·25
At follow-up				
Age (years)	5·17 (4·12–6·18) n=78	5·08 (4·10–6·11) n=78	6·55 (4·33–8·16) n=133	<0·001
Weight (kg)	15·6 (13·0–18·1) n=77	15·0 (12·5–19·8) n=78	17·1 (13·5–21·7) n=133	<0·001
Height (cm)	104·5 (95·5–114·5) n=78	105·5 (95·5–117·5) n=78	112·5 (100·0–124·0) n=133	<0·001
MUAC (mm)	158 (148–172) n=77	158 (148–174) n=77	160 (146–176) n=133	0·89
Used medication in past 3 days	10/78 (13%)	15/78 (19%)	13/132 (10%)	0·15
History of hospitalization	29/78 (37%)	35/78 (45%)	42/132 (32%)	0·16
Has ≥1 older sibling	52/78 (67%)	58/78 (74%)	83/133 (62%)	0·20
Animals at house	22/78 (28%)	32/78 (41%)	49/133 (37%)	0·23
Breastfeeding >18 months	58/78 (74%)	60/78 (77%)	106/133 (80%)	0·66
Mother answered survey	68/78 (87%)	66/78 (85%)	107/133 (80%)	0·42
BCG scar present	64/75 (85%)	70/77 (91%)	107/132 (81%)	0·16
Scar >5mm in children with a scar	13/64 (20%)	22/70 (31%)	26/107 (24%)	0·32
Age of BCG vaccination (days)	14 (1–51) n=76	8 (1–55) n=76	15 (1–58) n=132	0·62

Data are number (%) or median (10th – 90th centiles). MUAC=mid-upper arm circumference. VAS=vitamin A supplementation. DTP3=3rd dose of DTP vaccination, expected at 14 weeks according to national policy. OPV3=3rd dose of oral polio vaccine expected at 14 weeks. p value is for no difference between the three groups (Kruskal-Wallis).

Supplementary table 3: Anthropometry at birth, demography, and environmental factors and risk of atopy

	Atopy	Odds ratio (95% CI)	p value
Anthropometry at enrolment of BCG trial			
Maternal MUAC (n=247)			
Per 1 mm increase		0.98 (0.97-0.99)	0.003
MUAC			
Per 1mm increase (n=279)		1.00 (0.96-1.04)	0.87
Weight			
Per 1 kg increase (n=281)		2.84 (0.66-12.2)	0.16
Length			
Per 1 cm increase (n=280)		1.17 (0.98-1.39)	0.08
At follow up			
Sex			
Male	18/123 (15%)	1.00 (ref)	
Female	21/158 (13%)	1.01 (0.48-2.14)	0.97
Twins			
No	32/225 (14%)	1.00 (ref)	
Yes	7/56 (12%)	0.90 (0.30-2.69)	0.85
Age			
3-4 years	11/95 (12%)	1.00 (ref)	
5-6 years	24/152 (16%)	1.38 (0.59-3.22)	0.46
7-9 years	4/34 (12%)	0.95 (0.26-3.47)	0.94
		p value for trend	0.79
Breastfeeding			
≤18 months	8/62 (13%)	1.00 (ref)	
>18 months	31/219 (15%)	1.36 (0.56-3.29)	0.49
Season of birth			
December-May	21/160 (13%)	1.00 (ref)	
June-November	18/121 (15%)	1.25 (0.60-2.59)	0.55
Follow up period			
17 th October-22 nd November	19/134 (14%)	1.00 (ref)	
23 rd November-13 th December	20/147 (14%)	0.90 (0.43-1.89)	0.78
Older siblings			
None	17/92 (18%)	1.00 (ref)	
1-2	18/140 (13%)	0.72 (0.32-1.61)	0.43
≥3	4/49 (8%)	0.45 (0.12-1.66)	0.23
		p value for trend	0.19
Animals at house			
No	31/181 (17%)	1.00 (ref)	
Yes	8/100 (8%)	0.44 (0.19-1.00)	0.05
Chickenpox infection			
No	22/190 (12%)	1.00 (ref)	
Yes	17/91 (19%)	1.83 (0.86-3.88)	0.12

Data are number (%). MUAC=Mid-upper arm circumference. Odds ratios for anthropometry at birth are adjusted only for age and sex. Other estimates are adjusted for all other factors at follow up in the table.

Supplementary table 4: Two-by-two analyses of the effect of early BCG vaccination and vitamin A supplementation on skin prick reaction ≥ 2 mm

	All Children n=281	Early BCG group n=145	Control group n=136	Odds ratio Early BCG vs. control (95% CI)	p value
SPT ≥ 2 mm in all children* (n=281)	108/281 (38%)	54/145 (37%)	54/136 (40%)	0.89 (0.54–1.47)	0.65
Stratified by VAS					
VAS	36/76 (47%)	19/37 (51%)	17/39 (44%)	1.42 (0.56–3.57)	0.46
No VAS	72/205 (35%)	35/108 (32%)	37/97 (38%)	0.74 (0.41–1.33)	0.31
Odds ratio					
VAS vs. no VAS (95% CI)	1.76 (0.98–3.13)	2.46 (1.13–5.32)	1.27 (0.56–2.87)		
p value	0.06	0.02	0.56	interaction between BCG and VAS	0.24
SPT ≥ 2 mm restricted to children in VAS trial# (n=152)	62/152 (41%)	31/77 (40%)	31/75 (41%)	0.99 (0.50–1.97)	0.98
Stratified by VAS					
VAS	36/76 (47%)	19/37 (51%)	17/39 (44%)	1.33 (0.53–3.37)	0.55
Placebo	26/76 (34%)	12/40 (30%)	14/36 (39%)	0.71 (0.25–2.00)	0.51
Odds ratio					
VAS vs. Placebo (95% CI)	1.74 (0.87–3.45)	2.38 (0.90–6.25)	1.27 (0.47–3.44)		
p value	0.12	0.08	0.64	interaction between BCG and VAS	0.38

Data are number (%). VAS=vitamin A supplementation. *VAS vs. placebo and those not enrolled in vitamin A trial. #VAS vs. placebo. Odds ratios are adjusted for age and sex.

Supplementary table 5: Effect of vitamin A supplementation on atopy stratified by BCG randomization group and early or late DTP

	All children (n=281)		Early BCG group (n=145)		Control group (n=136)	
	Atopy	OR (95% CI)	Atopy	OR (95% CI)	Atopy	OR (95% CI)
Atopy (skin prick reaction ≥ 3 mm)						
DTP ≤ 8 weeks of age						
No vitamin A (n=144)	16/144 (11%)	1.00 (ref)	7/80 (9%)	1.00 (ref)	9/64 (14%)	1.00 (ref)
Vitamin A (n=59)	16/59 (27%)	3.19 (1.29–7.91)	8/27 (30%)	5.15 (1.57–16.82)	8/32 (25%)	2.14 (0.61–7.47)
DTP >8 weeks of age						
No vitamin A (n=61)	5/61 (8%)	1.00 (ref)	0/28 (0%)	1.00 (ref)	5/33 (15%)	1.00 (ref)
Vitamin A (n=17)	2/17 (12%)	1.50 (0.23–9.93)	2/10 (20%)	∞ (1.60– ∞)*	0/7 (0%)	0 (0–3.54)*

Data are number (%). OR=odds ratio adjusted for age, sex, and BCG randomization group (except BCG stratified analyses). DTP=1st dose of DTP vaccination expected at six weeks of age. *Cornfield approximation.

Supplementary table 6: Effect of vitamin A supplementation on symptoms of atopic disease

	Vitamin A (n=78)	No vitamin A (n=211)	Odds ratio (95% CI)	p value
Eczematous rash	3/78 (4%)	4/208 (2%)	1·96 (0·34–11·3)	0·45
Wheeze ever	27/78 (35%)	49/210 (23%)	1·77 (0·97–3·20)	0·06
Wheeze in past 12 months	19/77 (25%)	25/210 (12%)	2·45 (1·20–4·96)	0·01
Exercise induced wheeze	2/78 (3%)	9/211 (4%)	0·59 (0·13–2·78)	0·51
Dry cough after exercise	18/77 (23%)	44/211 (21%)	1·15 (0·61–2·17)	0·67
Nocturnal cough	5/77 (6%)	16/211 (8%)	0·88 (0·30–2·56)	0·81
Reaction to food	9/78 (12%)	16/208 (8%)	1·58 (0·68–3·69)	0·29

Data are number (%). Odds ratio adjusted only for BCG randomization group due to low number of events in some groups.