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# BMJ Open

## Protocol for Pertussis Immunisation and Food Allergy (PIFA): A case-control study of the association between pertussis vaccination in infancy and the risk of IgE-mediated food allergy

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ABSTRACT:

**Introduction:** Atopic diseases, including food allergy, have become a predominant cause of chronic illness among children in developed countries. In Australia, a rise in hospitalisations among infants coded as anaphylaxis to foods coincided with the replacement of whole-cell pertussis vaccine (wP) with subunit acellular pertussis vaccine (aP) on the national immunisation schedule in the late 1990s. Atopy is characterised by a tendency to mount T-helper type 2 responses to otherwise innocuous environmental antigens. Compared to infants who receive aP as their first pertussis vaccine, those who receive wP appear less likely to mount T-helper type 2 immune responses to either vaccine or extraneous antigens. We therefore speculate that removal of wP from the vaccine schedule contributed to the observed rise in IgE-mediated food allergy among Australian infants.

**Methods and analysis:** This is a retrospective individually-matched case-control study among a cohort of Australian children born from 1997 to 1999, the period of transition from wP to aP vaccines; we include in the cohort children listed on Australia’s comprehensive population-based immunisation register as having received a first dose of either pertussis vaccine by 16 weeks old. 500 cohort children diagnosed as having IgE-mediated food allergy at specialist allergy clinics will be included as cases. Controls matched each case by date and jurisdiction of birth and regional socioeconomic index will be sampled from the immunisation register. Conditional logistic regression will be used to estimate odds ratio ( $\pm 95\%$ CI) of receipt of wP (versus aP) as the first vaccine dose among cases compared to controls.

**Ethics and dissemination:** The study is approved by all relevant human research ethics committees: Western Australia Child and Adolescent Health Services (2015052EP), Women’s and Children’s Hospital (HREC/15/WCHN/162), Royal Children’s Hospital

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(35230A), Sydney Children's Hospital Network (HREC/15/SCHN/405). Outcomes will be disseminated through publication and scientific presentation.

**Trial Registration Details:** NCT02490007, status: enrolling

### Strengths and limitations of this study

- The transition from wP to aP in Australia represents a natural experiment of the effect of infant pertussis vaccination on subsequent food allergy on a scale not achievable by a randomised controlled trial.
- The study is nested within Australia's comprehensive, prospective, population-based immunisation register, meaning that ascertainment of vaccination status is likely to be accurate and any misclassification is likely to be non-differential for cases and controls.
- We included only cases diagnosed as IgE-mediated food allergy in specialist tertiary allergy clinics.
- Not all children diagnosed with IgE-mediated food allergy had a formal food challenge, the gold-standard for diagnosis.
- We assume that the probability of receipt of Wp as the first pertussis vaccine dose (rather than Ap) was dependent on date and jurisdiction of birth, but independent of any other risk factors for food allergy. It is not possible to verify this assumption is valid.



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those containing the acellular pertussis antigen (aP). Use of aP began to replace that of wP in the mid 1990s and by early 1999 most scheduled childhood pertussis vaccines were aP(12). In the United States and in the United Kingdom, a temporal association between the phasing out of wP vaccines and the increase in hospitalisations for food allergy is not clear (unpublished data, personal communication) although coding of hospitalisation records for allergies may be both insensitive and non-specific so any associations could be easily obscured. In light of the contrasting immunological effects of wP compared with aP (reviewed below), it is plausible that a causal relationship underlies the ecological association observed in Australia.

**The immunological basis of atopic disease:** A reciprocal relationship exists between T-helper cell type 1 and type 2 (Th1 and Th2) immunity due to cross-regulation of their respective effector cell populations(13). The balance between Th1 and Th2 is established during early infancy. Atopy is caused by a dysregulation of this balance in the developing immune system, characterised by an immune phenotype that is heavily biased toward Th2-immunity or ‘Th2-polarised’, the consequent over-production of IgE to one or more allergens, and IgE-mediated inflammation.

The developing immune system appears most susceptible to Th2 polarisation in the critical early months after birth. This period is pivotal in the transition from the Th1-suppressed/Th2-dominant phenotype needed to avoid rejection in utero, to a more Th1/Th2 balanced phenotype. Newborns are exposed to an array of new antigenic proteins from infection and other natural environmental exposures, including gut flora and food components. The development of tolerance to these natural exposures represents an early challenge to the developing immune system. It appears the development of tolerance can be influenced by a



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stimulating ligands present in wP. These effects manifest especially among children with evidence of an underlying Th2-skewed phenotype(21, 22).

**The broader immuno-modulating properties of pertussis:** The Th2 polarising effect of the initial dose of aP appears to extend beyond vaccine-specific responses; there may be a significant “bystander” effect with up-regulation of circulating IgE to a broad range of antigens following subsequent doses of aP. Importantly, these Th2-stimulatory effects appear to extend to food allergens(24), especially in early infancy when immune memory against allergens is most susceptible to programming(25, 26). The administration of additional pertussis vaccine doses in later childhood in children primed with aP-only is associated with frequent injection-site reactions. In Australia, this contributed to the removal of the 18 month old pertussis booster given in 2003, which in turn led to reduced protection against pertussis among pre-school aged children(27). These adverse reactions have been linked to the presence of high levels of vaccine-specific, Th2-polarised, Th-memory cells(28). Children primed with aP-only also exhibit high titres of total IgE, including tetanus-specific(29, 30) and pertussis-specific IgE(29). These responses are rarely observed among children who have received wP containing vaccines, including those who have received mixed vaccine schedules of wP and aP(31). A recent study of a birth cohort examined the effect of delaying aP vaccination on atopic outcomes. This study found that delay of aP by 1 month resulted in a reduction in eczema in infants and use of eczema medications(32). To date, no other studies have found a relationship between aP vaccines and allergy. A study from the 1990s found no significant difference in overall rates of skin prick test reactivity or allergic disease among young Swedish children who received wP compared to those who received aP(33). In that study population the frequency of food allergy was low (~2%) compared to that observed among contemporary Australian infants. A more recent prospective birth cohort of children

from the Isle of Wight also failed to find any difference in the frequency of atopic outcomes among children receiving wP versus aP (34).

Based on these observations, we speculate that removal of wP from the infant vaccine schedule has contributed to the observed rise in IgE-mediated food allergy among Australian infants.

AIM

To assess the possible food allergy-preventive benefit of using wP compared with aP for pertussis vaccination in childhood.

OBJECTIVES AND OUTCOME MEASURES

Primary objective

To determine if Australian children born between 1997 to 1999 (inclusive) who received wP as their first pertussis vaccine dose in infancy were less likely to subsequently develop IgE-mediated food allergy compared with contemporaneous children who received aP as their first pertussis vaccine dose.

Secondary objectives

(I) To determine if Australian children born in the years 1997 to 1999 (inclusive) who received at least one dose of a wP pertussis vaccine at any age were less likely to subsequently develop IgE-mediated food allergy compared with contemporaneous children who received only aP pertussis vaccines.

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(II) To determine if Australian children born in the years 1997 to 1999 (inclusive) who received wP pertussis vaccines exclusively were less likely to subsequently develop IgE-mediated food allergy compared with contemporaneous children who received only aP pertussis vaccines.

### Pre-specified hypotheses

*Primary Hypothesis:* Among Australian children with documented evidence of receiving a pertussis vaccine before age 16 weeks, a record of receipt of a wP vaccine as dose one is less common than aP vaccine among those subsequently diagnosed with food allergy compared to date of birth, socioeconomic index and jurisdiction -matched cohort controls.

*Secondary Hypothesis I:* Among Australian children with documented evidence of receiving a pertussis vaccine before age 16 weeks, a record of receipt of at least one dose of wP vaccine at any age is less common than aP vaccine at all ages among those subsequently diagnosed with food allergy compared to date of birth, socioeconomic index and jurisdiction-matched cohort controls.

*Secondary Hypothesis II:* Among Australian children with documented evidence of receiving a pertussis vaccine before age 16 weeks, a record of receipt of one or more wP vaccine doses exclusively (i.e. no aP vaccine) is less common than one or more aP vaccine doses exclusively (i.e. no wP vaccines) among those subsequently diagnosed with food allergy compared to date of birth, socioeconomic index and jurisdiction matched cohort controls.

### METHODS AND ANALYSIS

**Study Design**

This is a retrospective individually-matched case-control study of Australian children born during the period of transition from use of wP containing pertussis vaccines to aP containing pertussis vaccines (year of birth 1997-1999 inclusive) and who are registered on the Australian Immunisation Register (AIR; prior to 2016 known as the Australian Childhood Immunisation Register, ACIR) and who have received their first dose of pertussis vaccine before age 16 weeks. Cases are drawn from private allergy clinics and allergy clinics associated with tertiary teaching hospitals around Australia. Five hundred children identified as having IgE mediated food allergy, from reviewing case histories against standardised inclusion criteria, will be enrolled. Cohort controls will be drawn from a de-identified database of the Australian Immunisation Register held by the National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases. Each case will be individually matched with up to 10 cohort controls based on year of birth, socioeconomic index and jurisdiction.

Project coordination and epidemiological analysis is conducted from the Wesfarmers Centre of Vaccines and Infectious Diseases at the Telethon Kids Institute Western Australia.

**Primary Outcome Measure**

The primary outcome is IgE-mediated food allergy diagnosed by a registered specialist paediatric allergist.

**Definition of exposure of interest (vaccination)**

For the primary analysis, exposure is defined as Australian Immunisation Register (AIR) documented receipt of either a wP containing vaccine or receipt of an aP containing vaccine

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as the first vaccine against pertussis and given before age 16 weeks (strictly <112 days) irrespective of any subsequent pertussis vaccinations.

For the secondary analyses, exposure is defined as either:

(I) AIR documented receipt of *one or more* doses of wP at any age or only aP containing vaccines for all pertussis vaccinations. All other sequences of vaccines will be coded as non-applicable and excluded from the analysis.

(II) AIR documented receipt of *only* wP containing vaccine for all pertussis vaccinations or *only* aP containing vaccines for all pertussis immunisations. All other sequences of vaccines will be coded as non-applicable and excluded from the analysis.

Vaccination status for each case and its corresponding matched controls is referenced from the age of allergy diagnosis in the case.

## Study Setting

Cases are drawn from private allergy clinics and from allergy clinics associated with tertiary teaching hospitals in the Australian states of New South Wales, Victoria, South Australia and Western Australia; matched cohort controls are selected from among children registered as resident in the same state and recorded as having received at least one pertussis vaccine dose on the population-based vaccine register (AIR).

## Participant Identification

### *Eligibility criteria*

All cases and cohort controls must be registered on AIR as having had a first dose of any pertussis containing vaccine before age 16 weeks and during the period in which the transition from wP to aP vaccine occurred: 1<sup>st</sup> January 1997 to 31st December 1999.

*Overall description of trial participants*

To maximise the study efficiency, in the case identification has been concentrated among children born when aP accounted for between 25% to 75% of infant vaccine doses administered. The changeover from wP to aP occurred with slightly different timing in the different jurisdictions of Australia so the exact dates vary across jurisdictions. In the first instance, cases have been identified from among clinic attendees in NSW, Victoria, Queensland and Western Australia who are AIR-registered and born from 1<sup>st</sup> October 1998 to 30<sup>th</sup> June 1999. For South Australia, cases have been drawn from AIR-registered children born from 1<sup>st</sup> July 1997- 30<sup>th</sup> October 1998 owing to the earlier uptake of aP in that state. 500 cases in total will be enrolled. If fewer than 500 cases are identified from among children born within the initial birthdate range, the CI will expanded this birthdate range for NSW, Victoria, Queensland and Western Australia to 1<sup>st</sup> June 1998- 30<sup>th</sup> October 1999; for South Australia the birthdate range will expanded to 1<sup>st</sup> April 1997- 30<sup>th</sup> April 1999. If there are still insufficient eligible cases identified, the birthdate range will be progressively increased by 3 month increments in each direction for all jurisdictions until 500 cases are identified, or until the limits of 1<sup>st</sup> January 1997 and 31<sup>st</sup> December 1999 are reached (whichever occurs first).

*Case definition*

Cases are considered to have IgE-mediated food allergy on the basis of 1) a documented history of consistent clinical symptoms following ingestion of an implicated food, and 2) evidence of sensitisation to that food via either positive skin-prick test or elevated specific IgE to the implicated food, with onset after the first pertussis-containing vaccine but before age 15 years.

To meet the case definition of IgE-mediated food allergy, the case must satisfy BOTH:

- 1  
2  
3 1. The clinical notes or a clinical letter arising from the allergy consult explicitly  
4 documents the presence of one or more of the following features,:

- 5  
6 a. urticaria  
7  
8 b. angioedema  
9  
10 c. emesis  
11  
12 d. vocal hoarseness  
13  
14 e. persistent cough  
15  
16 f. wheeze  
17  
18 g. stridor  
19  
20 h. collapse  
21  
22 i. hypotension  
23  
24  
25

26 with onset of at least one feature within 1 hour of ingestion of the suspected food  
27  
28 where this can reasonably inferred from statements such as “immediate” or “within x  
29  
30 minutes” where x is <60.  
31  
32

33 AND

- 34  
35 2. Documented evidence of allergic sensitisation to the implicated food through

36 EITHER:

- 37  
38 a) specific IgE positive to suspected food (serum specific IgE >0.35KU/l) , or  
39  
40 b) positive skin prick test (SPT) to suspected food (wheal diameter >3mm)  
41  
42

43 where evidence of sensitisation must be at the time of consultation or within 6 months  
44  
45 after the clinical encounter.  
46  
47  
48  
49

50 Other acceptable terms:

51  
52 When reviewing case histories the below terms are considered synonymous:

- 53  
54 a. urticaria – hives, rash, welts  
55  
56  
57  
58  
59  
60

- b. angioedema - oedema, swelling of lips or eyes
- c. emesis- vomit or vomiting
- d. vocal hoarseness - horse voice, raspy voice
- e. persistent cough
- f. wheeze
- g. stridor
- h. collapse - faint, loss of consciousness
- i. hypotension - low blood pressure

The case definition has been agreed upon by specialist allergists associated with the study as consistent with international expert consensus for the definition of IgE-mediated food allergy.

*Determination of exposure (vaccination)*

The primary exposure of interest for case and cohort controls is the first received pertussis vaccine as recorded on the Australian Immunisation Register (AIR). The Australian vaccination schedule recommends three sequential priming vaccines against pertussis at approximately 2, 4 and 6 months of age. Cases and controls will have either received aP or wP together with tetanus (T) and diphtheria (D), either with or without hepatitis B (hepB) as part of commercial combined vaccine preparations. All children will have been eligible for booster doses of aP at age 18 months old and at age 4 years old.

ETHICS AND DISSEMINATION

This study was approved by human research ethics committees (HREC) in each jurisdiction. The PIFA study is approved by the Child and Adolescent Health Service Human Research Ethics Committee, Perth, Western Australia (Ref:2015052EP), Adelaide Women’s and

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Children's Hospital HREC, South Australia (Ref:HREC/15/WCHN/162), Royal Children's Hospital HREC, Victoria, Australia (Ref:35230A) and the Sydney Children's Hospital Network HREC, New South Wales, Australia(Ref: HREC/15/SCHN/405).

A waiver of consent was sought and approved on the basis that the study poses negligible risk to participants and that seeking individual consent for access to data was unfeasible and may lead to ascertainment bias.

## STATISTICS

### **Sample size and power considerations**

A study involving 500 sets of cases and controls, with 10 matched controls for each case, has 80% power to detect a 23% lower risk (odds ratio 0.77) of food allergy among children who received wP as their first dose of pertussis vaccine compared with those who received aP as their first dose. This assumes that: (i) 50% of cohort controls receive a first pertussis vaccine dose of wP on average; (ii) the correlation coefficient for exposure (first dose of wP) between cases and matched controls is 0.5; and (iii) a two-sided significance level of 5%. We believe that a smaller effect size will not influence vaccine policy.

### **Bias**

*Management of confounding:* Because routine vaccines in Australia are delivered almost exclusively via the National Immunisation Program, date of birth (and therefore date of vaccination) and jurisdiction are considered to be the only relevant factors associated with vaccine type received (wP versus aP), and therefore the only relevant potential confounders. We will minimise confounding by these factors by direct matching. In so far as the *type* of vaccine received is expected to be independent of ethnicity, sex, family size, birth order, pet ownership and all other factors putatively or known to be related to food allergy, there are

unlikely to be any other relevant confounders of the association between vaccine type and allergy; inability to match or adjust for other factors poses negligible threat to study validity.

*Minimising information bias:* The case definition is intended to be pragmatic and yet specific for IgE-mediated food allergy, excluding many non-allergic food reactions potentially misclassified as allergy by non-specialists and in hospital coding. For completeness, we will conduct an *a priori* sensitivity analyses which will include as cases only those children meeting the case definition who also have 1) challenge-proven food allergy and 2) evidence of sensitisation at or higher than the following levels: SPTs: 8 mm for cow milk, 7 mm for egg, and 8 mm peanut, or IgE serum responses: 15kU/L for cow milk, 7 kU/L for egg, 14kU/L for peanut. These are the documented levels for 95%PPV as defined by Sporik et al (35) and Sampson (36). Ascertainment of vaccination status will be from the AIR for both cases and controls and will not rely on either parental recall or recording in medical records. The AIR record of cases will only be ascertained after verification from the site PI that the case definition is fully met. Children with food allergy will be excluded as cases if they are not registered on AIR. There is no reason to expect that the accuracy or completeness of AIR should be different for cases and controls, so any inaccuracy is likely to be non-differential.

*Minimisation of selection bias:* Cases are sampled only from among children presenting to specialist-led private allergy clinics and allergy clinics at tertiary paediatric centres. To ensure correct classification of cases, we will not sample cases from non-tertiary Australian hospitals, from cases diagnosed and managed by non-specialists, or those without confirmation of sensitisation. We nonetheless expect cases will be generally representative of all Australian children with true IgE-mediated food allergy in the birth cohort. Cohort controls will be sampled from date-of-birth, jurisdiction and socioeconomic index matched children from the AIR, a comprehensive population-based register of all Medicare-registered children in Australia. Because cohort controls are sampled at random, they will provide an

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unbiased estimate of the vaccine status of the baseline source population for each case by date of birth, jurisdiction and IRSAD score (ABS-assigned socioeconomic index by postcode).

## Description of Statistical Methods

The study population characteristics will be summarised by case or control status using frequency and proportion for categorical or binary variables, means and standard deviations for symmetric continuous distributions and medians and inter-quartile ranges for asymmetric distributions. Conditional logistic regression will be used to perform hypothesis tests of the association between pertussis vaccine type (wP or aP) and IgE-mediated food allergy. Results will be summarised using odds ratios (OR's) and presented with associated 95% confidence intervals. Because controls will be sampled from the AIR irrespective of past or future case status, the odds ratio will be considered to be an unbiased estimator of the relative risk of food allergy among children receiving wP compared with aP vaccine.

## Analysis of Outcome Measures

*Primary analysis population:* All case and control individuals recorded on the AIR as receiving at least one dose of pertussis vaccine before age 16 weeks will be included in the primary analysis, irrespective of whether they received any further doses of pertussis vaccine.

*Primary analysis:* Conditional logistic regression will be used to evaluate the association between receipt of wP versus aP as the first pertussis vaccine and diagnosis of IgE-mediated food allergy. Direct matching will be on date of birth (+/- 7 days), jurisdiction (Australian state or territory), and IRSAD decile of the most recent Medicare-listed postcode (1<sup>st</sup> to 10<sup>th</sup>); no factors or interactions will be adjusted for in the *a priori* analysis.

For the primary analysis, a child’s exposure will be coded as *either*:

aP1: first pertussis vaccine dose of aP, with subsequent doses either wP or aP or none

*or*

wP1: first pertussis vaccine dose of wP, with subsequent doses either wP or aP or none

The comparison of primary interest will be between aP1 and wP1 vaccinated children.

*Secondary analyses population:* As for the primary analysis population, however the comparison of wP-only and aP-only vaccinated children (secondary analysis II) will exclude any children who received a mixture of aP and wP vaccines.

*Secondary analyses:* As for the primary analysis to evaluate the association between pertussis vaccines received (wP-only or mixed wP/aP in any combination, compared to aP-only) and IgE-mediated food allergy.

For secondary analyses: (I), a child’s exposure will be coded as either:

aP\_only: all pertussis vaccine doses as aP, none as wP

*or*

wP\_mix: at least 1 dose of wP at any age.

The comparison of interest is between aP-only and at least one dose of wP vaccine.

For secondary analyses: (II) a child’s exposure will also be coded as either:

aP\_only: all pertussis vaccine doses as aP, none as wP

*or*

wP\_only: all pertussis vaccine doses as wP, none as aP

The comparison of interest is between aP-only and wP-only vaccinated children. All mixed aP/wP vaccinated children will be excluded from the analysis. Table 1 provides a summary of the coding of exposure for the primary and secondary.

Any analyses other than those outlined above or in the sensitivity analyses and case subgroup analysis below will be declared as unplanned and *post hoc*.

Table 1

1 <sup>st</sup> dose Study eligibility requires first dose before age 16 weeks	2 <sup>nd</sup> dose	3 <sup>rd</sup> dose	Primary coding 1 <sup>st</sup> wP v 1 <sup>st</sup> aP	Secondary (1) coding Any wP v Only aP	Secondary (2) coding Only wP v Only aP
wP	missing	missing	wP1	wP_mix	wP_only
wP	wP	missing	wP1	wP_mix	wP_only
wP	wP	wP	wP1	wP_mix	wP_only
wP	aP	aP or missing	wP1	wP_mix	Not included
wP	aP or missing	aP	wP1	wP_mix	Not included
aP	wP or missing	wP	aP1	wP_mix	Not included
aP	wP	wP or missing	aP1	wP_mix	Not included
aP	aP	aP	aP1	aP_only	aP_only
aP	aP	missing	aP1	aP_only	aP_only
aP	missing	missing	aP1	aP_only	aP_only

Table 1- Summary of the coding of exposure for the primary and secondary analysis.

### Matching procedures

For each identified case, up to ten children will be randomly sampled without replacement from the AIR database from among all children born on the same day as the case +/- 7 days, and from the same jurisdiction and from a postcode with the same IRSAD decile (1<sup>st</sup> to 10<sup>th</sup>). Children will only be included once as a case. Each cohort control will be associated with one unique case. Consistent with the case-cohort method(37), sampling of controls will be from the register cohort without regard to case status (i.e. children will be eligible to be a control

irrespective of whether they are at any stage a case). In total up to 5,000 controls will be sampled. IRSAD deciles will be ascertained from ABS data from the 2011 census since it is likely that only the most recent Medicare postcode is available for the majority of the participants.

**Sensitivity analysis**

In the first instance, a sensitivity analysis will be performed on the case definition for the primary analysis based on confirmation of food allergy via a food challenge. Confirmation by food challenge is considered the gold standard for food allergy, but in clinical practice this has more usually been reserved for cases in which there is diagnostic uncertainty or to confirm resolution of the food allergy. If there is a significant change in the interpretation of the results based on the sensitivity analysis compared to the primary analysis, then further sensitivity analyses will also be performed for the secondary analyses.

As an additional sensitivity analysis, the case definition will require documentation of a skin prick test (SPT) wheal of 8 mm for cow milk, 7 mm for egg, or 8 mm peanut, or food-specific serum IgE of 15kU/L for cow milk, 7 kU/L for egg, or 14kU/L for peanut. These more conservative cut-offs correspond to almost 100% and 95% positive predictive value (PPV) of IgE-mediated allergy for the SPT and ss-IgE respectively.

**Case sub-group analysis**

To investigate the potential heterogeneity of responses following exposure to specific vaccine formulations, we will conduct an *a priori* subgroup analysis by acellular vaccine brand

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(excluding children who have received mixed acellular vaccine types). All other subgroup analyses will be declared as unplanned and *post hoc*.

## DISCUSSION

Allergy represents a significant disease burden in developed countries. The temporal association between an apparent increase in severe food allergy and the replacement of whole cell with acellular pertussis vaccine in Australia warrants further investigation. Food allergy is not only important in its own right, it is also associated with eczema and with asthma (4-fold increased risk) in later childhood(38, 39).

This study has been designed to comply with both the STROBE recommendations and also the more specific recommendations put forward by Sharpe et al(40) for case-cohort studies. The strengths of this study include objective allergy definition, and access to an established prospective population-based vaccine register for determination of vaccination status of both cases and cohort controls. Access to patient lists of allergist-diagnosed food allergy from large paediatric referral centres along with detailed prospectively collected immunisation records from the Australian Immunisation Register provide a unique opportunity to investigate a potential relationship between these events. The transition from wP to aP in Australia represents a natural experiment of the effect of infant wP on subsequent food allergy, on a scale unachievable by a randomised controlled trial.

The disadvantages of this study are that it is reliant on existent databases for which there is little way to assess the validity or accuracy of data entry. The AIR database has been lauded as a highly effective means of tracking vaccination in the Australian population, however there is evidence of under reporting of administered vaccines(41), and there has been no

validation of the quality of data entry into the database. Finally, we assume that the type of pertussis vaccine administered was dependent on jurisdiction and date of birth, but not otherwise dependent on factors which are also risk factors for allergy. While this assumption appears entirely reasonable we are not able to assess its validity.

**Ethics and dissemination:** The study is approved by all relevant human research ethics committees: Western Australia Child and Adolescent Health Services (2015052EP), Women’s and Children’s Hospital (HREC/15/WCHN/162), Royal Children’s Hospital (35230A), Sydney Children’s Hospital Network (HREC/15/SCHN/405). Outcomes will be disseminated through publication and scientific presentation.

**Trial Registration Details:** NCT02490007, status: enrolling

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Competing interests statement.  
Authors have no competing interests.

STROBE Statement—Checklist of items that should be included in reports of *case-control studies*

	Item No	Recommendation
<b>Title Pg 1 and abstractPg3-4</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstractPg1 (b) Provide in the abstract an informative and balanced summary of what was done and what was foundPg3-4
<b>Introduction</b>		
Background/rationalePg6-9	2	Explain the scientific background and rationale for the investigation being reportedPg6-9
ObjectivesPg9-10	3	State specific objectives, including any prespecified hypothesesPg9-10
<b>Methods</b>		
Study designPg11-12	4	Present key elements of study design early in the paperPg11-12
Setting Pg12-13	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Pg12-13
ParticipantsPg12-16	6	(a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controlsPg12-15 (b) For matched studies, give matching criteria and the number of controls per casePg16, Pg 20-21
Variables Pg as detailed	7	Clearly define all outcomesPg9, 11, exposuresPg11-12, predictors Pg16, potential confoundersPg16, and effect modifiers Pg16. Give diagnostic criteria, if applicablePg13-15
Data sources/ measurementPg11-13	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement)Pg 11-13. Describe comparability of assessment methods if there is more than one groupNA
BiasPg 16-18	9	Describe any efforts to address potential sources of biasPg16-18
Study sizePg16	10	Explain how the study size was arrived atPg16
Quantitative variablesPg18-20	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and whyPg18-20
Statistical methodsPg18	12	(a) Describe all statistical methods, including those used to control for confoundingPg16-22 (b) Describe any methods used to examine subgroups and interactionsPg18-20 (c) Explain how missing data were addressedPg20 (d) If applicable, explain how matching of cases and controls was addressedPg20-21 (e) Describe any sensitivity analysesPg21
<b>Results NA- Protocol only</b>		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest

Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(b) Report category boundaries when continuous variables were categorized
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
<b>Discussion Protocol only</b>		
Key results	18	Summarise key results with reference to study objectives NA
LimitationsPg22	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential biasPg16&22
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence NA
Generalisability	21	Discuss the generalisability (external validity) of the study resultsPg22-23
<b>Other information</b>		
FundingPg26	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is basedPg26

\*Give information separately for cases and controls.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.