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Title: Modes of Infant Feeding and the Risk of Childhood Asthma: a Prospective Birth Cohort Study

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Abstract: Objective: The role of breastfeeding in the prevention of asthma is uncertain. Previous studies have not differentiated between direct breastfeeding and expressed breast milk. We aimed to determine whether different modes of infant feeding are associated with childhood asthma.

Study Design: We studied 3296 children in the CHILD birth cohort. The primary exposure was infant feeding mode at 3 months, reported by mothers and categorized as: direct breastfeeding only, breastfeeding with some expressed breast milk, breast milk and formula, or formula only. The primary outcome was asthma at 3 years, diagnosed by trained healthcare professionals.

Results: At 3 months, the distribution of feeding modes was 27% direct breastfeeding, 32% breastfeeding with some expressed breast milk, 26% breast milk and formula, 15% formula. At 3 years, 12% of children were diagnosed with possible or probable asthma. Compared to direct breastfeeding, any other mode of infant feeding was associated with an increased risk of asthma. These associations persisted after adjusting for maternal asthma, ethnicity, method of birth, infant sex, gestational age, and daycare attendance (some expressed breast milk: aOR=1.43, 95%CI: 1.04-1.97; breast milk and formula: aOR=1.56, 95%CI: 1.12-2.18; formula only: aOR=1.79, 95%CI: 1.23-2.61). Results were similar following further adjustment for total breastfeeding duration and respiratory infections.

Conclusions: Modes of infant feeding are associated with asthma development. Direct breastfeeding is most protective compared to formula feeding, while indirect breast milk confers intermediate protection. Policies that facilitate and promote direct breastfeeding could have significant impact on the primary prevention of asthma.

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Dr. Denise M. Goodman
Associate Editor, The Journal of Pediatrics

May 13, 2017

Re: Revised Manuscript #2017412
"***Modes of infant feeding and the risk of childhood asthma: a prospective birth cohort study***"

Dear Dr. Goodman,

Thank you for reviewing our manuscript. We appreciate the comments from the Reviewers and Editors. We have enclosed a point-by-point response and revised our manuscript accordingly. We have also completed the Revision Checklist.

We believe these revisions have strengthened our manuscript, and hope you will now find it suitable for publication in *The Journal of Pediatrics.*

Sincerely,

Meghan Azad, PhD
Assistant Professor, Department of Pediatrics and Child Health, University of Manitoba
Research Scientist, Children's Hospital Research Institute of Manitoba

Encl. Revision Checklist and Point-by-Point Response to Reviewers.

Revision Checklist and Point-by-Point Response to Reviewers (Manuscript #2017412)

Revision Checklist

X Please upload the list of Study Group members (full name, degree, affiliation, city, and state) as a separate manuscript file (Word document), making sure to change the file description to "Appendix; online."

X Check that all reporting guidelines are met for the type of study reported in this manuscript (equator-network.org). If they are not, provide a detailed explanation in the point-by-point response for the Editors' consideration.

X Make your revision as short as possible; focus the Discussion and remove all redundancy between sections of text and between illustrations and text.

X The manuscript must be double-spaced throughout, including the references.

X Make sure that your Abstract is <250 words. For an Original Article, the Abstract must be structured as explained in our Guide for Authors (http://www.jpeds.com/authorinfo).

X The Objective of the Abstract should put the study in context with the current literature (i.e., what is new, not textbook background information) and reflect the purpose of the study. Background information should not be included, and it must begin with the hypothesis that is being tested or the question being asked (e.g., "To assess …," "To evaluate …").

X Please provide the industry-relation, funding source(s), and/or any conflicts of interest for Atul Sharma, referenced in the Acknowledgments section.

X Because The Journal adheres to Vancouver style, all references must list the first six authors before "et al," and all issue numbers in parentheses must be deleted.

X_Be sure that figures, if any, are submitted in TIFF, BMP, JPEG, GIF, PNG, EPS, PPT, or DOC format. Line art (black lines on a white background) must be created at 1,000 dpi. Combination line art (eg, line art with gray fill patterns) must be created at 1,200 dpi. Black and white or color photographs must be created at 300 dpi. Figure legends must appear on a separate page from the figures.

X Online only tables and figures, if any, should be submitted "as usual" through EES. Indicate what should be published online only in: (1) your point-by-point response; (2) EES, type "Figure x; online only" in the file description field when you upload the files; and (3) manuscript text, add behind the reference to the figure or table going online only "(Table x; online)." Do not renumber online only tables and figures or label them as "supplemental."

Point-by-Point Responses to Editor and Reviewers

Editor

1) I share the reviewers' concerns regarding ensuring that confounders and analysis are appropriate (please see reviewer comments).

We have addressed these concerns in our responses to Reviewers 1 and 2 below by evaluating additional potential confounders, and clarifying our modeling strategy.

2) The abstract states that the primary outcome is "physician diagnosis of asthma", but page 5 of methods only alludes to a "focused history and physical examination by…healthcare professionals", which could be a number of disciplines. Please clarify.

The methods are correct. We have elaborated on the disciplines (physician or nurse or clinical research associate) and amended the abstract to match.

3) I was unable to access Reference 13. Please clarify how the current submission differs substantively from that manuscript. From the title it appears to link breastfeeding with asthma in the first year, whereas the current paper links breastfeeding with asthma at age 3.

This reference was In Press at the time of our original submission. It has since been published (on May 2, 2017), and is available here: http://erj.ersjournals.com/content/49/5/1602019. We have updated the citation accordingly.

The Editor is correct; our recent publication examined breastfeeding (duration/exclusivity, but not feeding *mode*) and wheezing in the first year, whereas the current manuscript evaluates 1) feeding *mode* and 2) asthma at 3 years. These are unique and important distinctions because 1) the importance of feeding *mode* has been understudied to date, and 2) only a proportion of wheezing infants go on to develop asthma. Whereas no attempt was made to diagnose asthma at 1 year in the CHILD Study, the current manuscript reports on 'asthma' at 3 years, based on a directed history and assessment by a healthcare professional, and (new in the revised manuscript) a modified 'Asthma Predictive Index'.

Reviewer #1:

Thanks for your interesting contribution on the topic of breastfeeding and asthma from your birth cohort! The manuscripts reads very well and follows a clear outline.

Thank you for acknowledging the importance of this research topic. We have addressed your specific concerns below.

1) In the results section, there are two instances where the sentence reads like a "table caption"-type sentence ("The distribution of infant…" and "Associations of infant…") - please rephrase to actually describe the results, and after having finished describing a concept/a table/a result cite the table/figure.

We have rephrased these sentences as requested.

2) The discussion is very long, I suggest to limit it to a clearly structured and focused text, preferentially with subsection headings guiding the reader through the text.

We have created subheadings in the Discussion and have made the text more succinct.

3) Primary outcome/exposure. As far as I can see, there was only one outcome and one exposure assessed. I strongly (!) suggest to assess at least one or two additional outcome (definitions), based on whatever original information on and around asthma you collected at age 3 years. The exposure could as well be explored in more detail, e.g. by differentiating between different breastfeeding durations? This exposure-outcome pair is the core (and actually sole) focus of your analysis, warranting a thorough sensitivity analysis at least by varying exposure and outcome definitions to the extent possible with your available data.

Thank you for this suggestion. We have added an alternative outcome definition: modified Asthma Predictive Index, a more stringent definition that identifies children with 'possible or probable asthma' who also have parental history of asthma, or diagnosed atopic dermatitis, or atopic sensization by skin test. The new outcome variable is defined in the Methods section and results have been added to the 'Sensitivity Analysis' section of Table 3.

While we agree that duration of breastfeeding is important, and we have accounted for it in our sensitivity analysis, we have elected to focus our main analyses on the unique and understudied question of feeding *mode*.

4) T1: suggest to contrast against those lost to follow-up (change right-most column)

This change has been made, and the corresponding text has been adjusted accordingly.

5) T2: what is the added information compared to T1? Suggest to delete or merge this table (in general, it is easier to read/understand if you have clearly labelled samples throughout the manuscript, give them a "label", e.g. "complete data sample", "breastfeeding data sample", ….)

Agreed. We have combined these two tables and adjusted the text accordingly. We have also updated and simplified the flow diagram in Figure 1, and revised the first paragraph of Results to consistently report on the 2534 children with complete feeding and asthma data.

6) T3: could be plotted together with T1? Maybe too cluttered than. Report all (!) p-values to the same decimal (if p-values are necessary here at all)

We have merged T3 and T4 and reported all p-values to 3 decimal places. These revisions help to clearly identify the potential confounders (variables associated with both feeding mode and asthma). Thank you for the suggestion.

7) T4: Was this information used to decide which covariates to put into the model? It seems this was done irrespective of the data shown here (pls explain, also to the reader)

Yes, this information was used to select covariates. This is demonstrated more clearly in the revised Table 2 (combining original Tables 3 and 4). We have also clarified the selection of covariates in the Methods as follows:

"Logistic regression was used to investigate associations between modes of infant feeding and asthma. First, potential confounders (listed above) were tabulated against infant feeding mode and asthma. Those found to be significantly associated with both feeding mode and asthma ($p<0.05$ by chi-squared test) were subsequently included in logistic regression models. Regression models were also adjusted for three established asthma risk factors selected *a priori* (infant sex, maternal ethnicity and maternal asthma)."

8) T5+T6: please put in one table, limit footnotes to those really necessary to understand the table, don't repeat the methods. Pls limit the title text to a considerable length.

Done. Tables 5 and 6 are combined in the new Table 3.

9) F2: …shows information completely doubled from the tables (and actually: the text). Pls avoid double quotes of data/analysis, only key data should appear in the text and either a table or a figure, never in 3 places (not counting the abstract and conclusion). If shown as a figure, pls put the frequency above the effect estimates (in line with the analytic approach taken), provide 95%-CI for the frequency estimates, don't show a "data item/point" for the reference, the reference line can be labelled though.

Yes, Figure 2 shows information from the Tables and this information is also reported in the text. We believe this is appropriate, since it is the main finding of the paper. The Figure provides a visual representation of this key finding, while the Table provides the corresponding numerical results (adjusted effect estimates and confidence intervals) *alongside* the crude effect estimates and sensitivity analyses. This presentation in Table form facilitates comparison of crude vs. adjusted results, and main results vs. sensitivity analyses.

We have modified the text to be less repetitive, by excluding the crude ORs. We prefer to keep the frequencies below the effect estimates in Figure 2, but we have made the following requested changes: added the 95%CI for frequency estimates, removed the 'data point' for the reference, and labeled the reference line.

We appreciate your thoughtful input and valuable suggestions, which have helped strengthened our paper. Thank you!

Reviewer #2:

Klopp et al present a well-written, interesting and novel analysis on modes of breastfeeding at 3 months of age and the risk of asthma at 3 years in the Canadian CHILD cohort. This is a population based birth cohort of 3311 children recruited antenatally, of which 2635 had complete information (exposures /outcomes /confounders) for this analysis. The methods describe analysing both those with complete information on all variables used as well as using the entire cohort after imputation of missing variables (based on several baseline characteristics). They found a protective effect for direct breastfeeding when compared to all other feeding modes. However, they also found that receiving some expressed breast milk was more protective for asthma than receiving some formula, which were both more protective than being entirely formula fed. This is an important study which may help to explain some of the conflicting information on breastfeeding and the risk of asthma.

Thank you for acknowledging the significance of this research. We agree that our study addresses an important and timely topic, and identifies a knowledge gap requiring further investigation. We have addressed your specific concerns below.

Methods

1) Exposure definition

The exposure is perhaps subject to some misclassification if measured at 3 months, does this mean it includes feeding practices up to the age of 3 months?

We have clarified the exposure definitions in the Methods section as follows:

"Modes of feeding were reported by mothers at three months and infants were classified in four categories: 1) breast milk only – all direct breastfeeding (no expressed milk or formula from birth to three months); 2) breast milk only – some expressed breast milk (received some breast milk expressed with a pump before three months, but no formula); 3) formula and breast milk (formula introduced before three months, but still receiving some direct or expressed breast milk at three months); 4) formula only (not receiving any breast milk at three months)."

We have also acknowledged in the discussion that: "the frequency and timing of expressed milk feeding within the first 3 months was not reported, potentially leading to some exposure misclassification for feeding mode 'at 3 months'."

(Note: we originally referred to expressed milk being consumed 'in the last 2 weeks'. However, this period could actually refer to the 2 weeks prior to the questionnaire OR the 2 weeks before the mother stopped pumping. Therefore it is more accurate to refer to these infants as having EVER received expressed milk, and we have modified our exposure definition accordingly.)

2) Outcome definition

At 3 years as acknowledged by the authors it is notoriously difficult to separate asthma from viral wheeze.

The methods stated that both "possible or probable asthma" were defined as "asthma" at 3 years. Were these categories of possible and probable able to be separated and did the associations differ for those outcomes?

We agree that it is challenging to define and diagnose asthma at 3 years and have acknowledged this in our 'limitations' section:

"While our "asthma" diagnosis was based on a structured history and physical examination by trained healthcare professionals, we acknowledge that diagnostic uncertainty is an important concern at this age. Further research will be required to establish associations with confirmed asthma later in childhood…"

We decided not to separate "possible" from "probable" asthma at this age due to issues of diagnostic uncertainty (differing tendencies to diagnose possible vs. probable across different study sites) and to conserve power. However, we have now added a secondary outcome of modified Asthma Predictive Index, and similar associations are seen for this outcome.

3) What is not clear to me is how the final set of confounders were chosen for the models. Models should not be adjusted for variables that are only related to the exposure or the outcome but for those variables which could be common cause confounders (related to both). So, although the authors have stated they adjusted for known asthma risk factors (infant sex, maternal ethnicity and maternal asthma), all of these except perhaps infant sex could also be common cause confounders. The other confounders included were apparently related to both exposure and outcome (method of birth, gestational age and daycare attendance). Other common cause confounders that could be considered are maternal smoking in infancy, number of siblings, measures of SES, study site. Maternal smoking during infancy may have a greater impact on breastfeeding behaviour and may be different to maternal smoking measured during pregnancy- do the authors have this variable?

We have clarified our modeling strategy in the Methods as follows:

"Logistic regression was used to investigate associations between modes of infant feeding and asthma. <u>First, potential confounders (listed above) were tabulated against infant feeding mode and asthma. Those found to be significantly associated with both feeding mode and asthma ($p<0.05$ by chi-squared test) were subsequently included in logistic regression models.</u> Regression models were <u>also</u> adjusted for three established asthma risk factors <u>selected *a priori*</u> (infant sex, maternal ethnicity and maternal asthma)."

We agree that infant sex, maternal ethnicity and maternal asthma could be common cause confounders. Our results in Table 2 support this. We also agree that maternal smoking, siblings and SES could be confounders. We had already explored these factors using the following variables: maternal smoking in pregnancy, first born, and maternal education. All were significantly associated with infant feeding mode, but not with asthma at 3 years. We have now expanded or exploration of these potential confounders as follows (see Table 2):

- Added maternal smoking during *infancy*.
- Replaced binary 'first born' variable with a more detailed 3-category variable 'number of older siblings' (0, 1 or 2+).
- Added home ownership as a second measure of SES, and clearly stated that maternal education and home ownership are measures of SES (see Methods and Tables 1 and 2).

All of the above measures are strongly associated with infant feeding mode, but not with asthma at 3 years. Therefore, they were not included in the multivariate analysis.

4) Mediation analyses using early life infections may provide support for the microbiome hypothesis proposed to be behind the found association. There is no apparent mediation by respiratory tract infections when included in the model for the sensitivity analysis (table 6 online) although this is a very simple and sometimes inaccurate way to assess potential mediation. Did the authors consider a mediation analysis?

While we agree that we have not fully explored mediation and these analyses may be insightful, they are beyond the scope of the current manuscript. We intend to pursue such analyses in the future, after refining our measures of respiratory infections (work is underway to combine questionnaire data, healthcare records and nasal swabs to categorize the severity of respiratory infections and identify specific pathogens). We have revised the following statement in the discussion:

"Further research will be required to establish associations with confirmed asthma later in childhood and to determine the underlying mechanisms (for example, through formal mediation analyses accounting for early life infections classified by type, timing and severity, and analysis of breast milk bioactivity following expression and storage."

5) In contrast to seeing little change in the effect estimates with addition of respiratory infections in the sensitivity analysis, there is some evidence (from table 6 online) that the length of total breastfeeding may be partially mediating the found relationship between infant feeding mode and asthma. This may indicate either that the length of breastfeeding is important or that other SES factors related to the ability to breastfeed for longer may be confounding the association.

True. While it is difficult to determine the relevance of this small change, we have modified the reporting of this result as follows: "Sensitivity analyses adjusting for ~~total breastfeeding duration and~~ frequent respiratory infections yielded similar results, while adjustment for total breastfeeding duration resulted in slightly attenuated associations."

Discussion

6) The proposed hypothesis for the found association relates to immunologically active constituents of breast milk which may not preserve well in expressed breast milk and may be respond acutely at the time of breastfeeding. Have the authors also considered an alternate hypothesis involving the presence of potential toxins in the equipment used to collect and feed babies expressed breast milk. Chemicals implicated may

include those used to make up plastic components (Biphenols, phthalates, PFAS) along with cleaning agents used (eg triclosan).

Great point! We have added this hypothesis to our Discussion: "Expressed milk could also contain asthmogenic chemicals used in the manufacturing or cleaning of breast pumps or storage containers, including phthalates, bisphenols, or triclosan."

We appreciate your suggestions, which have helped strengthen our paper. Thank you!

Edited by DG and WFB

Modes of Infant Feeding and the Risk of Childhood Asthma: a Prospective Birth Cohort Study

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* List of additional members of the CHILD Study Investigators is available at www.jpeds.com (Appendix)

Comment [MM1]: Copyeditor: Include this in the footnote

Key Words: breastfeeding, pumped breast milk, asthma

~~**Document Data:** 2647; 3 Tables, 2 Figures~~

~~**Funding:** This research was f~~Funded by the Canadian Respiratory Research Network and the Allergy, Genes and Environment Network of Centres of Excellence, and supported by the Canadian Institutes of Health Research. ~~These entities had no role in the design or conduct of the study.~~The authors declare no conflicts of interest.

Comment [MM2]: Author: Please provide grant numbers if applicable.

~~**Conflict of Interest:** The authors declare no conflicts of interest.~~

~~**Contributors' Statement:**~~

~~Drs Sears, Mandhane, Turvey, Subbarao and Becker conceived of the cohort design, managed study recruitment and oversaw data collection.~~

~~Dr Lefebvre managed the CHILD Study database.~~

~~Ms Vehling cleaned and collated data to generate breastfeeding variables.~~

~~Drs Klopp and Azad conceived and conducted the analysis, and drafted the manuscript.~~

~~All co-authors provided feedback and approved the final version and take responsibility for the integrity of the data and the accuracy of the data analysis.~~

~~**Abstract**~~

Objective~~:~~ ~~The role of breastfeeding in the prevention of asthma is uncertain. Previous studies have not differentiated between direct breastfeeding and expressed breast milk. We aimed to~~To determine whether different modes of infant feeding are associated with childhood asthma, including differentiating between direct breastfeeding and expressed breast milk.

Study ~~D~~design~~:~~ We studied 3296 children in the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort. The primary exposure was infant feeding mode at 3 months, reported by mothers and categorized as: direct breastfeeding only, breastfeeding with some expressed breast milk, breast milk and formula, or formula only. The primary outcome was asthma at 3 years, diagnosed by trained healthcare professionals.

Results~~:~~ At 3 months, the distribution of feeding modes was 27% direct breastfeeding, 32% breastfeeding with some expressed breast milk, 26% breast milk and formula, 15% formula. At 3 years, 12% of children were diagnosed with possible or probable asthma. Compared to direct breastfeeding, any other mode of infant feeding was associated with an increased risk of asthma. These associations persisted after adjusting for maternal asthma, ethnicity, method of birth, infant sex, gestational age, and daycare attendance (some expressed breast milk: aOR=~~1.43~~1.64, 95%CI: ~~1.04-1.97~~1.12-2.39; breast milk and formula: aOR=~~1.56~~1.73, 95%CI: ~~1.12-2.18~~1.17-2.57; formula only: aOR=~~1.79~~2.14, 95%CI: ~~1.23-2.61~~1.37-3.35). Results were similar following further adjustment for total breastfeeding duration and respiratory infections.

Conclusions~~:~~ Modes of infant feeding are associated with asthma development. Direct breastfeeding is most protective compared to formula feeding, while indirect breast milk confers intermediate protection. Policies that facilitate and promote direct breastfeeding could have ~~significant~~ impact on the primary prevention of asthma.

Comment [GDD3]: Author: These are your main results from Table 3. The figures you quoted in the submitted abstract were for the column from the sensitivity analysis including multiple imputation for missing data.

~~Introduction~~

Breast milk is widely known to be the optimal source of infant nutrition. The importance of breastfeeding is well recognized for infants' short-term health with respect to growth, immune function, and gastrointestinal health.[1] In addition to these immediate clinical benefits, there are potential long-term advantages that are realized after the breastfeeding period. An extensive body of literature suggests that breastfeeding may contribute to protection against autoimmune, malignant, and inflammatory diseases, including allergic diseases and asthma.[1–5] However, very few studies distinguish between *breastfeeding*, where the infant suckles directly at the mother's breast, and *consumption of human milk*, which can be expressed and fed from a bottle. This is an important distinction because an increasing number of mothers are providing expressed breast milk to their infants.[6,7] For example in the United States, where there is no national policy for paid maternity leave and the average mother returns to work after just 10 weeks,[8] over 25% of nursing mothers regularly provide expressed breast milk to their infants.[9]

~~Asthma is the most common chronic health problem in childhood, affecting approximately 10% of American children.~~[10] While several studies,[5,11,12] including our own,[13] have found that breastfeeding is protective against asthma or wheezing disorders, a recent meta-analysis found that evidence for this association was inconsistent across studies, with high heterogeneity (I^2= 63% across 29 studies) related to differences in study designs and settings.[14] This inconsistency may also be related to differences in infant feeding modes, which are known to vary widely between countries,[15] but are generally not documented in epidemiologic studies.

To date, only one study has examined respiratory health among infants fed direct breast milk versus bottled breast milk;[16] Soto-Ramirez et al~~.~~ found that any mode of infant feeding that

included formula or expressed breast milk conferred an increased risk for coughing/wheezing episodes by one year of age, compared to direct breastfeeding. A proposed mechanism for this association is the alteration of breast milk components, such as bioactive proteins and microbiota, during the expression and storage of breast milk.[17–19] In addition, during active infection in the nursing infant, direct breastfeeding is thought to trigger an increased immune response in the lactating mother leading to a transfer of protective factors to her relatively immunocompromised offspring.[20] Direct contact through breastfeeding also transmits potentially protective maternal skin microbes,[21] and the physical exercise associated with suckling at the breast is thought to improve airflow and increase lung capacity.[22] However, the potential impact of expressed breast milk on childhood asthma development remains unknown as no studies have examined this association beyond the first year of life.

Using prospective data from the national Canadian Healthy Infant Longitudinal Development (CHILD) population-based birth cohort,[23] we undertook a study to determine the association of infant feeding modes in the first 3 months of life with asthma development by age 3 years. We hypothesized that any mode of feeding that included expressed breast milk or formula would be associated with an increased risk of asthma compared to direct breastfeeding.

Methods

~~*Study design*~~

This study involved 3296 infants from ~~the general cohort of~~ the CHILD study,[23] a general ~~population~~ population-based national birth cohort that recruited pregnant women from Toronto, Winnipeg, Edmonton, and Vancouver from 2009-2012. The eligibility requirements for enrollment included women at least 18 years of age in their third trimester of pregnancy with

proficiency in English and residing within reasonable proximity to a recruitment centre. Exclusion criteria for the CHILD study were: children with major congenital anomalies, born preterm (less than 35+6 week), of multiple births, or resulting from in-vitro fertilization. Written informed consent was obtained by caregivers at enrollment and the study was approved by the Human Research Ethics Boards of the Universities of Alberta, British Columbia, Manitoba, Toronto and McMaster University.

~~Exposure: infant feeding mode at 3 months~~

Modes of feeding were reported by mothers at three months and infants were classified in four categories: 1) breast milk only – all direct breastfeeding (no expressed milk or formula from birth to three months); 2) breast milk only – some expressed breast milk (received some breast milk expressed with a pump before three months, but no formula); 3) formula and breast milk (formula introduced before three months, but still receiving some direct or expressed breast milk at three months); 4) formula only (not receiving any breast milk at three months).

~~Outcomes: possible or probable asthma diagnosis at 3 years~~

A semi-structured assessment of asthma was performed at 3 years of age. The diagnosis of asthma was made following a focused history and physical examination by a limited number of well-trained healthcare professionals (2 or 3 physicians, nurses or clinical research associates in each site) and classified for this analysis as 'possible or probable asthma' or 'no asthma'. In a sensitivity analysis, we evaluated "modified Asthma Predictive Index" as an alternative outcome, adapted from Guilbert at al.[24] and defined as a diagnosis of possible or probable asthma plus one

of: diagnosed atopic dermatitis,[23] positive skin prick test to any allergen (wheal diameter ≥2mm greater than the response to the negative control)[23], or parental history of diagnosed asthma (self-reported by parents).

Potential confounders

Infant sex, gestational age, method of birth, maternal age, and number of older siblings (parity) were documented from hospital records. Maternal ethnicity, history of asthma, and tobacco smoking during pregnancy and infancy were self-reported by standardized questionnaire. Maternal education and home ownership were also self-reported during pregnancy, and assessed as measures of socioeconomic status (SES). Daycare attendance at one year was defined as spending seven or more hours a week with at least three other children at a location away from home. Total duration of any breastfeeding (infant age at breastfeeding cessation) and number of respiratory infections (colds) were documented from maternal questionnaires completed at 3, 6, 12, 18 and 24 months postpartum.

Statistical analysis

Logistic regression was used to investigate associations between modes of infant feeding and asthma. First, potential confounders (listed above) were tabulated against infant feeding mode and asthma. Those found to be significantly associated with both feeding mode and asthma (~~p~~*P*<~~0~~.05 by chi-squared test) were subsequently included in logistic regression models. Regression models were also adjusted for three established asthma risk factors selected *a priori* (infant sex, maternal ethnicity, and maternal asthma). Results are presented as crude and adjusted odds ratios (OR, aOR) with 95% confidence intervals (CI). Analyses were conducted for the 2534 children with complete data for infant feeding, asthma diagnosis, and essential

Comment [MM4]: Copyeditor: Please format P values per Journal style throughout

covariates (**Figure 1**; available at www.jpeds.com~~, online~~). Children who were lost to follow up or had incomplete feeding data (N=762) were similar to those with complete data (N=2534) with respect to infant feeding patterns, maternal asthma, and child asthma (**Table ~~1~~I**). Mothers of children with complete data were more likely to be ~~Caucasian~~ white and have higher SES, and less likely to smoke. To address potential bias from incomplete data and loss to follow up, a sensitivity analysis was performed in the full cohort following multiple imputation of missing feeding, asthma, and covariate data. Multiple imputation (n=20 imputed datasets) was performed with fully conditional specification (chained equations) using all essential covariates plus the following auxiliary variables: maternal age, parity, and postsecondary education, history of prenatal smoke exposure, and study site. Additional sensitivity analyses were performed to evaluate an alternative outcome definition (modified Asthma Predictive Index, defined above), and to adjust for respiratory infections (among all infants) and breastfeeding duration (excluding infants in the 'formula only' group). All analyses were performed using SAS version 9.4 (SAS Institute, Cary, North Carolina).

Comment [MM5]: Copyeditor: Please format Table numbers per Journal style throughout (Roman numerals)

Results

Among 2534 infants with complete feeding and asthma data, the distribution of feeding modes at 3 months was: 60% breast milk only (27% all direct breastfeeding, 33% with some expressed breast milk), 26% breast milk and formula, and 14% formula only (**Table 1**). By 3 years of age, 319 (12.6%) were diagnosed with possible or probable asthma and 217 (8.6%) had a positive modified Asthma Predictive Index. Risk factors for possible or probable asthma diagnosis included: male sex, gestational age <37 weeks, maternal asthma, caesarean section delivery, and daycare attendance by one year of age (**Table 2**). Number of siblings, maternal education, and

maternal smoking were not significantly associated with asthma.

Mode of infant feeding was associated with several maternal and infant characteristics (**Table 2)**. ~~Direct~~ Exclusively breast milk feeding breastfeeding was associated with higher maternal education, Asian and Caucasian ethnicity, vaginal delivery, and gestational age ≥39 weeks. Firstborn infants received less direct breast milk and more expressed milk compared to those with older siblings, while the proportion receiving formula was similar regardless of birth order. Mothers who smoked were less likely to provide direct or expressed breast milk and more likely to provide formula. Infants who attended daycare were less likely to receive direct breast milk exclusively. ~~There was a trend towards more formula feeding and less direct breastfeeding among mothers with asthma.~~

Comment [GDD6]: Author: Changed to "exclusively breast milk feeding" rather than "direct breastfeeding" for these factors. Table 2 indicates higher percentages for some expressed breast milk than for direct breastfeeding for many of these.

Infant feeding mode was significantly associated with possible or probable asthma diagnosis at 3 years (**Table 3)**. Compared to direct breastfeeding, any mode of infant feeding that included expressed milk or formula was associated with an increased risk of possible or probable asthma diagnosis **(Figure 2)**. The lowest prevalence (8.8%) was observed among infants who received direct breast milk only. Prevalence was higher among infants receiving some expressed breast milk (12.5%) or breast milk and formula (14.9%), and was highest among exclusively formula fed infants (15.8%). Associations persisted after multiple imputation of missing data and adjustment for infant sex, maternal asthma, ethnicity, method of birth, daycare attendance, and gestational age (some expressed breast milk: aOR=1.43, 95%CI: 1.04-1.97; breast milk and formula: aOR=1.56, 95%CI: 1.12-2.18; formula only: aOR=1.79, 95%CI: 1.23-2.61). Similar patterns of association were found for the alternative outcome definition of modified Asthma Predictive Index (**Table 3**). Sensitivity analyses adjusting for frequent respiratory infections

Comment [GDD7]: Author: the values are depicted with multiple imputations for missing data, but that is a sensitivity analysis, not the primary findings. Please justify this depiction or consider using the main findings.

yielded similar results, while adjustment for total breastfeeding duration resulted in slightly attenuated associations **(Table 3)**.

Discussion

~~*New findings*~~

Our research in the population-based CHILD birth cohort indicates that any mode of infant feeding other than direct breastfeeding is associated with an increased likelihood of possible or probable asthma by 3 years of age. Compared to infants who received direct breast milk only, those who received some expressed milk had a 43% increased ~~risk~~ odds of this diagnosis, and those who received only formula had a 79% increased ~~risk~~odds. These associations were independent of established maternal, socioeconomic and environmental risk factors.

Comment [GDD8]: Author: please present primary findings instead of a sensitivity analysis

~~*Comparison to other studies*~~

Although several studies have examined the association between infant feeding and asthma,[5,11,12,14,25] ~~ours is the first to~~the present study distinguishes between direct breastfeeding and expressed breast milk. Our results suggest that feeding mode differences could help explain the apparently inconsistent results observed in "breastfeeding" studies across different populations and settings. Our study also extends recent findings by Soto-Ramirez et al~~.~~, who reported that American infants fed bottled breast milk or formula have an increased risk of coughing and wheezing episodes by one year of age compared to those who were directly breastfed.[16] Finally, our results are consistent with recent evidence that feeding expressed breast milk is associated with increased odds of otitis media[26] and rapid infant weight gain[27], compared to direct breastfeeding.

~~*Postulated mechanisms*~~

There are several possible explanations for the apparently differential effects of direct breastfeeding and expressed breast milk. One involves the alteration of breast milk components, such as immune cells, cytokines and microbiota, during the expression and storage of breast milk.[17–19] For example, freezing or processing human milk has been shown to diminish its antioxidative properties,[18] decrease vitamin levels, and reduce immunoglobulin A activity.[19] Expressed milk could also contain asthmogenic chemicals used in the manufacturing or cleaning of breast pumps or storage containers, including phthalates, bisphenols, or triclosan.[28] Another hypothesis is that when a nursing infant is sick, direct breastfeeding triggers an increased immune response in the lactating mother to provide her infant with a more effective immunologic defense.[20] Riskin et al~~.~~ showed that white blood cells and cytokines were increased in breast milk collected from mothers when their nursing infants were infected, even when the mothers themselves were asymptomatic.[20] While the mechanism explaining this phenomenon is not fully understood, there appears to be a bidirectional exchange of immune factors between mother and infant during direct breastfeeding.

In addition to the bioactive factors in breast milk, the physical act of breastfeeding may also play a role in asthma prevention. There is increasing evidence that commensal bacteria are essential to health,[21] and the direct skin-to-skin contact during breastfeeding provides a source of potentially protective maternal microbes to the nursing infant. Moreover, Ogbuanu et al~~.~~ showed that breastfed children have increased lung volumes by 10 years of age and attributed this advantage to the mechanical stimulus and “physical training” associated with sucking at the breast in early life.[22]

~~*Policy implications*~~

Breastfeeding is a societal responsibility that must be supported by government initiatives,[29] including paid maternity leaves that facilitate direct breastfeeding. In the American Infant Feeding Practices Study II, maternal employment was the strongest predictor of infant feeding mode between 1 and 5 months, with working mothers being twice as likely to provide expressed milk compared to those who were not working.[9] The US is one of only four countries worldwide without a national policy requiring paid maternity leave,[30] and even among countries that do have policies, only 53% meet the International Labor Organization's minimum recommendation of 14 weeks.[30] Our results suggest that programs and policies to support direct breastfeeding could have a meaningful impact on asthma prevention at the population level.

It is important to acknowledge that mothers often express milk due to logistic and physical barriers to breastfeeding, and these challenges should not be dismissed. Expression can help mothers continue to provide breast milk when breastfeeding is difficult or contraindicated, and during times of separation. Recognizing the many challenges new mothers encounter, our goal is not to discourage those who cannot provide direct breast milk exclusively, but rather to highlight the underappreciated differences between direct breastfeeding and expressed breast milk, guide further research, and inform societal policies and individual decisions about infant feeding.

~~*Study strength and limitations*~~

The major strengths of our study are the standardized prospective assessment of infant feeding in a large birth cohort, and the distinction of direct breastfeeding from expressed breast milk. Although 24% of eligible infants had incomplete feeding data or were lost to follow up, our

sensitivity analysis demonstrated that results were robust to multiple imputation of missing data. A limitation of our study is our inability to quantify the relative proportion of direct breast milk, expressed breast milk, and formula provided, which precludes evaluation of dose effects. Also, the frequency and timing of expressed milk feeding within the first 3 months was not reported, leading to potential exposure misclassification for feeding mode “at 3 months”. While our “asthma” diagnosis was based on a structured history and physical examination by trained healthcare professionals, we acknowledge that diagnostic uncertainty is an important concern at this age. Further research will be required to establish associations with confirmed asthma later in childhood and to determine the underlying mechanisms (for example, through formal mediation analyses accounting for early life infections classified by type, timing and severity, and analysis of breast milk bioactivity following expression and storage). Finally, as in all observational studies, we cannot exclude the possibility that our results may be influenced by unmeasured confounders, although we have controlled for multiple maternal and socioeconomic factors known to influence feeding practices and asthma development.

~~Conclusion~~

Our findings demonstrate that modes of infant feeding in the first 3 months of life are associated with a diagnosis of possible or probable asthma by 3 years of age. Compared to formula feeding, direct breastfeeding appears to be most protective, while expressed breast milk may confer intermediate levels of protection. Further research is warranted to confirm and explain the differential effects of direct breastfeeding and expressed breast milk. Meanwhile, policies that facilitate and promote direct breastfeeding could have a significant impact on the primary prevention of asthma.

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Figure Legends

Figure 1; online. CONSORT flow diagram.

Figure 2. Modes of infant feeding at 3 months and possible or probable asthma at 3 years in the CHILD cohort. *Odds ratios adjusted for infant sex, maternal asthma, ethnicity, method of birth, daycare attendance, and gestational age, with multiple imputation of missing data; lines represent 95% confidence intervals.

Online Appendix: Modes of infant feeding and the risk of childhood asthma: a prospective birth cohort study (Klopp et al. *J Pediatrics*)

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Table 1. Characteristics of CHILD Study dyads with complete versus incomplete data.

	Subjects with Complete Feeding and Asthma Data (N=2534)		Subjects with Incomplete Data (N=762)		
	n	(%)	n	(%)	p
Maternal asthma					
No	1961	(78.9)	557	(79.6)	0.569
Yes	526	(21.1)	143	(20.4)	
Missing	47		77		
Maternal ethnicity					
Asian	404	(16.0)	106	(14.8)	<0.001
Caucasian	1866	(74.0)	492	(68.5)	
First Nations	96	(3.8)	48	(6.7)	
Other	155	(6.1)	72	(10.0)	
Missing	13		59		
SES: Maternal education					
Did not complete post-secondary	550	(22.3)	191	(28.4)	0.001
Completed post-secondary	1914	(77.7)	481	(71.6)	
Missing	70		90		
SES: Home ownership					
Family owns home	1875	(76.0)	435	(64.8)	<0.001
Family rents home	591	(24.0)	236	(35.2)	
Missing	68		91		
Maternal smoking during pregnancy					
No	2305	(92.6)	581	(85.2)	<0.001
Yes	184	(7.4)	101	(14.8)	
Missing	45		80		
Maternal smoking during infancy					
No	2077	(94.6)	331	(89.0)	<0.001
Yes	118	(5.4)	41	(11.0)	
Missing	339		390		
Method of birth					
Vaginal	1877	(75.2)	536	(73.6)	0.296
Cesarean	620	(24.8)	192	(26.4)	
Missing	37		49		
Infant gestational age (weeks)					
<37	111	(4.5)	30	(4.2)	0.026
37-38	145	(5.8)	59	(8.2)	
38-39	420	(16.9)	137	(19.1)	
≥39	1815	(72.9)	493	(68.6)	
Missing	43		58		
Number of older siblings					
0	1369	(54.0)	392	(53.0)	0.520
1	838	(33.1)	260	(35.1)	
≥2	327	(12.9)	88	(11.9)	
Missing	0		22		
Infant sex					
Female	1187	(46.8)	376	(48.4)	0.319
Male	1347	(53.2)	401	(51.6)	
Missing	0		0		

Daycare attendance by 1 year					
No	1760	(80.5)	309	(81.3)	0.715
Yes	426	(19.5)	71	(18.7)	
Missing	348		397		
Feeding mode at 3 months					
Breast milk only: all direct breastfeeding	690	(27.2)	126	(25.6)	0.153
Breast milk only: some expressed breast milk	831	(32.8)	144	(29.3)	
Breast milk + Formula	659	(26.0)	137	(27.8)	
Formula only	354	(14.0)	85	(17.3)	
Missing	0		285		
Possible or probable asthma at 3 years					
No	2215	(87.4)	105	(92.9)	0.082
Yes	319	(12.6)	8	(7.1)	
Missing	0		664		
Modified asthma predictive index at 3 years					
Negative	2308	(91.4)	106	(94.6)	0.228
Positive	217	(8.6)	6	(5.4)	
Missing	9		665		

SES, socioeconomic status. Percentages reflect proportion of non-missing data. Comparisons by chi-squared test.

Table 2. Distribution of infant feeding modes at 3 months and prevalence of possible or probable asthma at 3 years, according to maternal and infant characteristics in the CHILD cohort.

		Feeding mode at 3 months									Possible or Probable Asthma at 3 years		
		Breast milk only: direct breastfeeding		Breast milk only: some expressed milk		Breast milk and formula		Formula only					
	N	n	(%)	n	(%)	n	(%)	n	(%)	p	n/N	(%)	p
Maternal asthma													
No	2338	650	(27.8)	747	(32.0)	623	(26.6)	318	(13.6)	0.055	235/2042	(11.5)	**0.004**
Yes	622	146	(23.5)	212	(34.1)	160	(25.7)	104	(16.7)		88/547	(16.1)	
Maternal ethnicity													
Asian	474	137	(28.9)	132	(27.8)	149	(31.4)	56	(11.8)	**<0.001**	60/419	(14.3)	0.152
Caucasian	2204	602	(27.3)	754	(34.2)	539	(24.5)	309	(14.0)		227/1936	(11.7)	
First Nations	123	25	(20.3)	32	(26.0)	31	(25.2)	35	(28.5)		11/105	(14.5)	
Other	199	45	(22.6)	54	(27.1)	70	(35.2)	30	(15.1)		28/169	(16.6)	
SES: Maternal education													
Did not complete post-secondary	673	164	(24.4)	172	(25.6)	166	(24.7)	171	(25.4)	**<0.001**	71/585	(12.1)	0.866
Completed post-secondary	2264	625	(27.6)	781	(34.5)	610	(26.9)	248	(11.0)		245/1976	(12.4)	
SES: Home ownership													
Family owns home	2183	589	(27.0)	734	(33.6)	583	(26.7)	277	(12.7)	**<0.001**	243/1937	(12.6)	0.632
Family rents home	754	200	(26.5)	219	(29.0)	193	(25.6)	142	(18.8)		74/626	(11.8)	
Maternal smoking during pregnancy													
No	2713	759	(28.0)	912	(33.6)	711	(26.2)	331	(12.2)	**<0.001**	296/2392	(12.4)	0.624
Yes	250	39	(15.6)	48	(19.2)	72	(28.8)	91	(36.4)		27/199	(13.6)	
Maternal smoking during infancy													
No	2326	648	(27.9)	807	(34.7)	595	(25.6)	276	(11.9)	**<0.001**	251/2144	(11.7)	0.896
Yes	150	33	(22.0)	15	(10.0)	42	(28.0)	60	(40.0)		15/124	(12.1)	
Method of birth													
Vaginal	2220	623	(28.1)	730	(32.9)	567	(25.5)	300	(13.5)	**0.003**	225/1960	(11.5)	**0.009**
Cesarean	756	176	(23.3)	232	(30.7)	216	(28.6)	132	(17.5)		100/649	(15.4)	
Infant gestational age (weeks)													
<37	125	22	(17.6)	39	(31.2)	44	(35.2)	20	(16.0)	**<0.001**	21/116	(18.1)	**0.007**
37-38	182	48	(26.4)	45	(24.7)	52	(28.6)	37	(20.3)		21/153	(13.7)	
38-39	516	116	(22.5)	150	(29.1)	157	(30.4)	93	(18.0)		71/438	(16.2)	
≥39	2144	610	(28.5)	725	(33.8)	527	(24.6)	282	(13.2)		211/1894	(11.1)	

Number of older siblings													
0	1640	345	(21.0)	611	(37.3)	432	(26.3)	252	(15.4)	**<0.001**	183/1418	(12.9)	0.645
1	1002	326	(32.5)	275	(27.4)	264	(26.3)	137	(13.7)		103/883	(11.7)	
≥2	382	144	(37.7)	89	(23.3)	100	(26.2)	49	(12.8)		41/346	(11.9)	
Infant sex													
Female	1430	417	(29.2)	468	(32.7)	329	(23.0)	216	(15.1)	**0.001**	131/1241	(10.6)	**0.008**
Male	1596	399	(25.0)	507	(31.8)	467	(29.3)	223	(14.0)		196/1406	(13.9)	
Daycare at 1 year													
No	1994	565	(28.3)	671	(33.7)	500	(25.1)	258	(12.9)	**0.026**	200/1821	(11.0)	**0.024**
Yes	482	110	(22.8)	156	(32.4)	139	(28.8)	77	(16.0)		65/438	(14.8)	

SES, socioeconomic status. Comparisons by chi-squared test. Significant associations (p<0.05) in **bold**.

Table 3. Association of infant feeding modes and possible or probable asthma at 3 years in the CHILD cohort.

Mode of infant feeding at 3 months	Possible or Probable Asthma at 3 years	Crude Association	Adjusted for Covariates[1]	Sensitivity Analyses (all Adjusted for Covariates[1])			
				Alternative Outcome Definition: Positive mAPI	Multiple Imputation (MI) of Missing Data	MI + Adjusted for Respiratory Infections[2]	MI + Adjusted for BF Duration (BF infants only)
	n/N (%) N=2534	OR (95%CI) N=2534	aOR (95%CI) N=2102	aOR (95%CI) N=2099	aOR (95%CI) N=3296	aOR (95%CI) N=3296	aOR (95%CI) N=2839
Breast milk only: direct breastfeeding	61/690 (8.8)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Breast milk only: some expressed breast milk	104/831 (12.5)	1.48 (1.06 - 2.06)	1.64 (1.12 - 2.39)	1.79 (1.13 - 2.83)	1.43 (1.04 - 1.97)	1.45 (1.05 - 2.01)	1.39 (1.02 - 1.92)
Breast milk and formula	98/659 (14.9)	1.80 (1.28 - 2.53)	1.73 (1.17 - 2.57)	1.76 (1.09 - 2.84)	1.56 (1.12 - 2.18)	1.61 (1.15 - 2.25)	1.44 (1.00 - 2.07)
Formula only	56/354 (15.8)	1.94 (1.32 - 2.86)	2.14 (1.37 - 3.35)	1.80 (1.03 - 3.14)	1.79 (1.23 - 2.61)	1.86 (1.28 - 2.71)	-

OR, odds ratio; CI, confidence interval; MI, multiple imputation.

[1]Adjusted for infant sex, maternal diagnosis of asthma, ethnicity, method of birth, daycare attendance, and gestational age.

[2]Frequent colds (4 or more in the first year of life).

Figure 1, online

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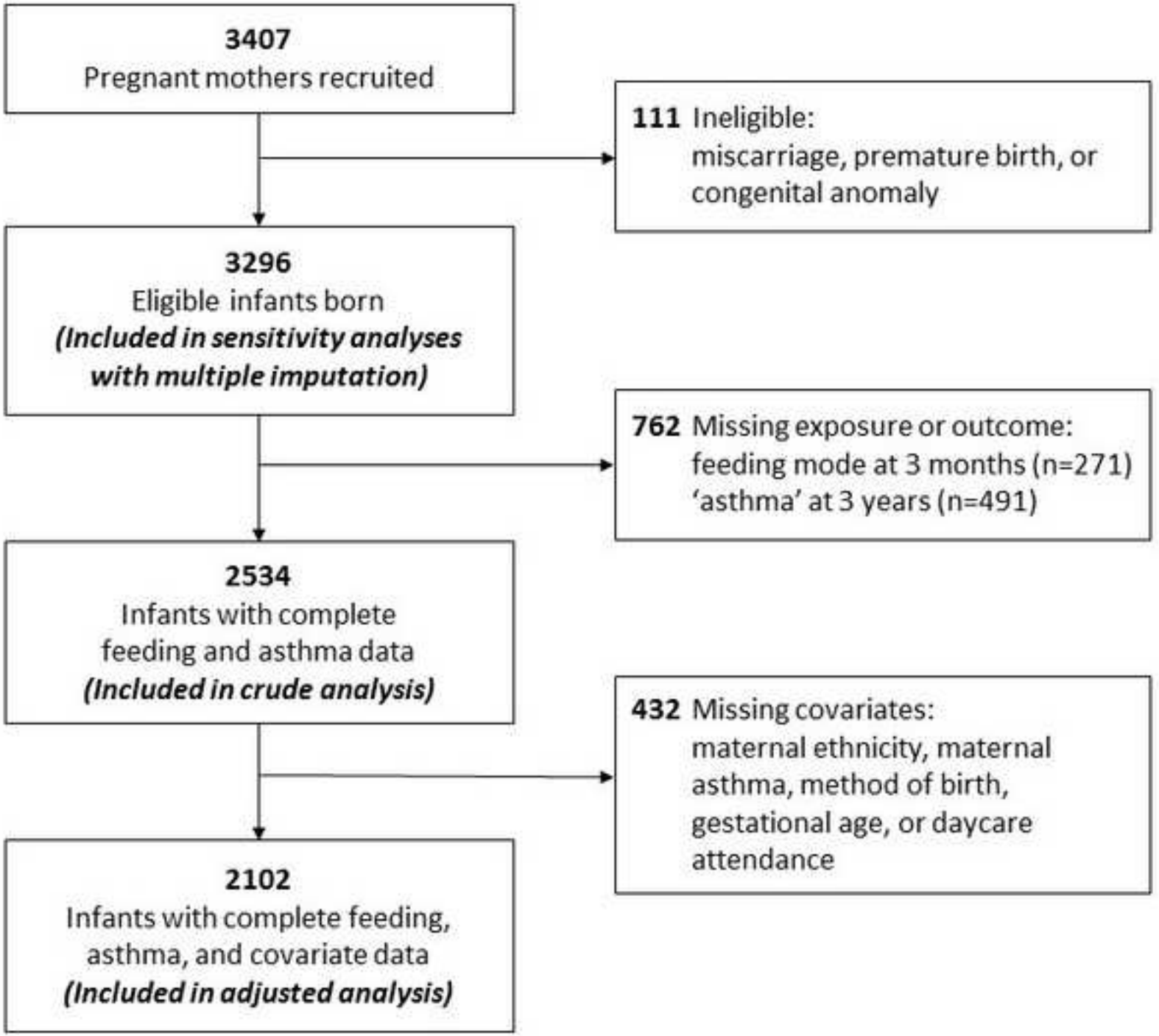

Figure 2

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