PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Breastfeeding and infant development in a cohort with sibling pair analysis: the Japan Environment and Children's Study
AUTHORS	Sanefuji, Masafumi; Senju, Ayako; Shimono, Masayuki; Ogawa, Masanobu; Sonoda, Yuri; Torio, Michiko; Ichimiya, Yuko; Suga, Reiko; Sakai, Yasunari; Honjo, Satoshi; Kusuhara, Koichi; Ohga, Shouichi

VERSION 1 – REVIEW

REVIEWER	Vohr, Betty Brown Univ, Pediatrics
REVIEW RETURNED	13-Nov-2020

GENERAL COMMENTS	Review: Breastfeeding and infant neurodevelopment in a cohort with sibling pair analysis: the Japan Environment and Children's Study This is a Nationwide prospective birth cohort study with sibling pair analysis of 77 119 children from 15 regional centers to assess the association between breastfeeding and infant neurodevelopment during the first year of life using sibling comparison. The subgroups for the discordant sibling analysis are much smaller but adequate. Abstract: clearly describes the study Methods Data (Jan 2011 –March 2014) was obtained from the Japan Environment and Children's Study (JECS), a nationwide cohort with > 100,000 term born children on which breast feeding data were collected monthly for the first year of life on both subjects and siblings. Data on feeding style, use of complementary foods, neurodevelopmental status, and affected diseases were collected at ages 1 and 6 months and every 6 months until the child was 6 years old, and then twice a year thereafter via self-reported questionnaires completed by the parents. Mothers completed a monthly feeding status questionnaire of their child when the child was 1 , 6, and 12 months old. This information included whether the child was breastfed, formula-fed, or both. Sibling matches were selected pairs who were discordant on the status of any breastfeeding or exclusive breastfeeding. Query, What was the range of # of years of age difference between the siblings ? Query: How was this measurement calculated ? Is it a yes/no response ? a graded response ? Is it by parent report ? "home speech stimulation at 1 month (whether a mother did or did not talk to her baby habitually" Outcome: Japanese translated version of the Ages and Stages Questionnaires completed by the parents. Cut-off scores were

	determined by using the same methodologies used in the original A&S version, based on available data at ages 6 months (n = 82 410) and 12 months. Comment: The Ages and Stages is a screening tool to measure developmetal skill domains. It does not measure neurodevelopment which implies a neurologic assessment. Comment: Suggest the title be modified s to state infant development rather than infant neurodevelopment. Again this is a screen that is completed by parents who may have their own biases. These findings need to be confirmed with diagnostic assessments administered by psychologists in a future investigation. Table 1 You list a significant percent of missing outcome data at 6 and 12 months. It is helpful to have your study design figure. Since all of the denominators differ, however, in Table 3 the legend should be clear of what it represents. Statistical Analysis: The large sample size permitted regression analyses adjusting for multiple neonatal, parental and SES characteristics including the home speech stimulation. Any breastfeeding continued during the first 6 months after birth was associated with reduced neurodevelopmental delay at ages 6 and 12 months. Any breastfeeding during the first 12 months was similarly associated with reduced delay at 12 months. Exclusive breastfeeding that continued during the first 3 months, but not the first 6 months, was associated with reduced neurodevelopmental delay at age 12 months (0.84 [0.80–0.88]). Comment: An unexpected finding that exclusive breast feeding to 6 months was not significantly assoc. with reduced developmental delay. Query: Isn't this contrary to prior findings of others related to exclusive breast feeding ? It would be of interest to expand discussion a bit on this finding. The persistent reduction in NDI after adjusting for siblings with discordant breast feeding status in this cohort is an important finding. Comment : The number of discordant pairs at 6 months was 389, and at 6 months 666. I would be helpful to include in your Study design figure since as it stands it does not depict the smaller sample size for the final important regression. Limitation: The developmental outcome as assessed by the Ages and Stages was completed by parents. A good first step. A standardized developmental assessment administered by a professional / PCP or psychologist/would provide more definitive results.

REVIEWER	Welch, Martha G. Columbia Univ Coll Phys
REVIEW RETURNED	21-Dec-2020

GENERAL COMMENTS	In their manuscript, "Breastfeeding and infant neurodevelopment in a cohort with sibling pair analysis: the Japan Environment and Children's Study," Sanefuji and co-authors have assessed the characteristics of breast feeding (duration) with developmental delay in a very large population of infants in Japan at 6 and 12 mos. This study may be suitable for publication if the authors can resolve the very large limitation as to what factors have contributed to cessation of breastfeeding. It is not known whether early

	cessation of breast feeding is a cause, consequence or correlate of developmental delay. This needs to be stated clearly in the introduction and discussed in the limitations section at a minimum. In addition the authors should cite the Family Nurture Intervention study that has demonstrated the role served by nurturing activities in infant development [1-8]. The authors should also discuss possible mechanisms involved with breastfeeding that could affect neurodevelopment, especially the connection between motivation and relational health (See [9]) [1] B. Beebe, M.M. Myers, S.H. Lee, A. Lange, J. Ewing, N. Rubinchik, H. Andrews, J. Austin, A. Hane, A.E. Margolis, M. Hofer, R.J. Ludwig, M.G. Welch, Family nurture intervention for preterm infants facilitates positive mother-infant face-to-face engagement at 4 months, Dev Psychol, 54 (2018) 2016-2031. [2] A.A. Hane, J.N. LaCoursiere, M. Mitsuyama, S. Wieman, R.J. Ludwig, K.Y. Kwon, V.B. J, J. Austin, M.M. M, M.G. Welch, The Welch Emotional Connection Screen: validation of a brief mother-infant relational health screen, Acta Paediatr, 108 (2019) 615-625. [3] A.A. Hane, M.M. Myers, M.A. Hofer, R.J. Ludwig, M.S. Halperin, J. Austin, S.B. Glickstein, M.G. Welch, Family nurture intervention improves the quality of maternal caregiving in the neonatal intensive care unit: evidence from a randomized controlled trial, J Dev Behav Pediatr, 36 (2015) 188-196. [4] S.W. Porges, M.I. Davila, G.F. Lewis, J. Kolacz, S. Okonmah-Obazee, A.A. Hane, K.Y. Kwon, R.J. Ludwig, M.M. Myers, M.G. Welch, Autonomic regulation of preterm infants is enhanced by Family Nurture Intervention, Dev Psychobiol, 61 (2019) 942-952. [5] M.G. Welch, J.L. Barone, S.W. Porges, A.A. Hane, K.Y. Kwon, R.J. Ludwig, R.I. Stark, A.L. Surman, J. Kolacz, M.M. Myers, Family nurture intervention in the NICU increases autonomic regulation in mothers and children at 4-5 years of age: follow-up results from a randomized controlled trial, PLoS One, In Press (2020). [6] M.G. Welch, J.L. Barone, S.W. Porges, A.A. Hane, K.Y. Kwon, R.J. Ludwig, R.I. Stark, A.L. Surman, J. Kolacz, M.M. Myers, Family nurture intervention in the NICU increases autonomic regulation in mothers and children at 4-5 years of age: Follow-up results from a randomized controlled trial, PLoS One, 15 (2020) e0236930. [7] M.G. Welch, M.R. Firestein, J. Austin, A.A. Hane, R.I. Stark, M.A. Hofer, M. Garland, S.B. Glickstein, S.A. Brunelli, R.J. Ludwig, M.M. Myers, Family Nurture Intervention in the Neonatal Intensive Care Unit improves social-relatedness, attention, and neurodevelopment of preterm infants at 18 months in a randomized controlled trial, J Child Psychol Psychiatry, 56 (2015) 1202-1211. [8] M.G. Welch, M.S. Halperin, J. Austin, R.I. Stark, M.A. Hofer, A.A. Hane, M.M. Myers, Depression and anxiety symptoms of mothers of preterm infants are decreased at 4 months corrected age with Family Nurture Intervention in the NICU, Arch Womens Ment Health, 19 (2016) 51-61. [9] R.J. Ludwig, M.G. Welch, How babies learn: The autonomic socioemotional reflex, Early Hum Dev, 151 (2020) 105183.

REVIEWER	Duffy, David QIMR Berghofer Medical Research Institute, Genetic Epidemiology, Molecular Epidemiology and Neurogenetics Laboratories

REVIEW RETURNED	10-Feb-2021

GENERAL COMMENTS	This seems to be an excellent resource for addressing these hypotheses. I will largely comment on analysis. For me, one threat to the conclusions is reverse causation, ie that subtle neurodevelopmental deficits might lead to mothers having difficulty breast feeding. Given a parental questionnaire is being used, rather than a clinical assessment, measurement might be imperfect, so developmental delay (DD) may not be reliably diagnosed. This might mean that undiagnosed DD at six months might precede cessation of breast feeding, with the DD is only diagnosed at, say, 12 months. The effects size are small enough that this might be a consideration. Discounting this possibility might be addressed by a longitudinal analysis of the data, since presumably repeat measurements on the same children can be identified (trajectories). For example, do delays at six months resolve by twelve months more often in children who were breast fed to twelve months? Does DD at six months predict children whose mother ceases breast feeding between six and twelve months? Were participants asked to retrospectively report when they stopped breast feeding? Rather than treating this as multiple correlated binary variables, a single length would be more informative. This would require an appropriate longitudinal linear mixed model - I would treat development scores directly as well as a dichotomized delay outcome (which would require a GLMM). Given that this is a well characterized cohort, I would not report odds ratios, but risk differences etc for DD. I would also like to see more results for the *confounders* in the sibling analysis, especially with respect to birth order. Does breast feeding of the first born predict a reduced risk of DD in the second born who was not breast fed? Maternal smoking seems moderately common - how many cases are there where the mother smoked only for one pregnancy, and how does this interact with the choice to breast feed longer? These kinds of tabulations could be supplementary, but are of great interest to me in terms of

	causation (recalling that residual confounding from strong but imperfectly measured constructs such as social class may not be completely adjusted for by just including parental education/income). In passing, in the non-family-based analysis, consider parent-child correlation in minor developmental conditions, say a mother and infant with ADHD at various stages, that might lead to difficulties with breast feeding. A definite strength of the matched-sibling design is dealing with these possibilities. The meta-analysis of Tseng et al, 2019 for example, claims 'children with ADHD received significantly higher rate of exclusive breastfeeding duration "under 3 months" but lower in "over 3 months" than controls' - again I am positing reverse causation as an alternative explanation here.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Dr. Betty Vohr, Brown Univ

Comments to the Author:

Review: Breastfeeding and infant neurodevelopment in a cohort with sibling pair analysis: the Japan Environment and Children's Study

This is a Nationwide prospective birth cohort study with sibling pair analysis of 77 119 children from 15 regional centers to assess the association between breastfeeding and infant neurodevelopment during the first year of life using sibling comparison. The subgroups for the discordant sibling analysis are much smaller but adequate.

Abstract: clearly describes the study

[Response] Thank you very much.

Methods

Data (Jan 2011 –March 2014) was obtained from the Japan Environment and Children's Study (JECS), a nationwide cohort with > 100,000 term born children on which breast feeding data were collected monthly for the first year of life on both subjects and siblings. Data on feeding style, use of complementary foods, neurodevelopmental status, and affected diseases were collected at ages 1

and 6 months and every 6 months until the child was 6 years old, and then twice a year thereafter via self-reported questionnaires completed by the parents.

Mothers completed a monthly feeding status questionnaire of their child when the child was 1, 6, and 12 months old. This information included whether the child was breastfed, formula-fed, or both. Sibling matches were selected pairs who were discordant on the status of any breastfeeding or exclusive breastfeeding.

Query, What was the range of # of years of age difference between the siblings?

[Response] We have added the information in **Table 4** (old Table 3).

Query: How was this measurement calculated? Is it a yes/no response? a graded response? Is it by parent report? "home speech stimulation at 1 month (whether a mother did or did not talk to her baby habitually"

[Response] It is just a 'yes/no' response. We have added the information in **Statistical analysis** section of **Methods**.

Outcome: Japanese translated version of the Ages and Stages Questionnaires completed by the parents. Cut-off scores were determined by using the same methodologies used in the original A&S version, based on available data at ages 6 months (n = 82 410) and 12 months.

Comment: The Ages and Stages is a screening tool to measure developmetal skill domains. It does not measure neurodevelopment which implies a neurologic assessment.

Comment: Suggest the title be modified s to state infant development rather than infant neurodevelopment.

Again this is a screen that is completed by parents who may have their own biases. These findings need to be confirmed with diagnostic assessments administered by psychologists in a future investigation.

[Response] Thank you for the sensible comment. We have changed "neurodevelopment" into "development" throughout the manuscript including the title.

Table 1 You list a significant percent of missing outcome data at 6 and 12 months.

It is helpful to have your study design figure.

[Response] We have added that information to **Figure 1**.

Since all of the denominators differ, however, in Table 3 the legend should be clear of what it represents.

[Response] We have revised it.

Statistical Analysis: The large sample size permitted regression analyses adjusting for multiple neonatal, parental and SES characteristics including the home speech stimulation.

Any breastfeeding continued during the first 6 months after birth was associated with reduced neurodevelopmental delay at ages 6 and 12 months. Any breastfeeding during the first 12 months was similarly associated with reduced delay at 12 months.

Exclusive breastfeeding that continued during the first 3 months, but not the first 6 months, was associated with reduced neurodevelopmental delay at age 12 months (0.84 [0.80–0.88]).

Comment: An unexpected finding that exclusive breast feeding to 6 months was not significantly assoc. with reduced developmental delay.

Query: Isn't this contrary to prior findings of others related to exclusive breast feeding? It would be of interest to expand discussion a bit on this finding.

The persistent reduction in NDI after adjusting for siblings with discordant breast feeding status in this cohort is an important finding.

[Response] As mentioned in the 4th paragraph of **Discussion**, the association of exclusive breastfeeding seems less consistent compared to any breastfeeding. We have added a possible reason referring to other reviewer's comments.

Comment: The number of discordant pairs at 6 months was 389, and at 6 months 666. I would be helpful to include in your Study design figure since as it stands it does not depict the smaller sample size for the final important regression.

[Response] Thank you very much for this valuable comment. We have added the information in **Figure 1**. In addition, we have noticed imprecise description in this part. "The number of discordant pairs increased from 43 (1.4%) pairs by (> at) the first month of life to 389 (12.5%) pairs by (> at) 6 months and 666 (21.4%) pairs by (> at) 12 months." The numbers represent those of discordant pairs at the age points.

Limitation: The developmental outcome as assessed by the Ages and Stages was completed by parents.

A good first step.

A standardized developmental assessment administered by a professional / PCP or psychologist/would provide more definitive results.

[Response] Thank you so much for the encouraging comments. The JECS already collected the data of a face-to-face standardized neurodevelopmental assessments at 2 and 4 years of age. We will analyze them in the next step.

Reviewer: 2

Dr. Martha G. Welch, Columbia Univ Coll Phys

Comments to the Author:

In their manuscript, "Breastfeeding and infant neurodevelopment in a cohort with sibling pair analysis: the Japan Environment and Children's Study," Sanefuji and co-authors have assessed the characteristics of breast feeding (duration) with developmental delay in a very large population of infants in Japan at 6 and 12 mos. This study may be suitable for publication if the authors can resolve the very large limitation as to what factors have contributed to cessation of breastfeeding. It is not known whether early cessation of breast feeding is a cause, consequence or correlate of developmental delay. This needs to be stated clearly in the introduction and discussed in the limitations section at a minimum. In addition the authors should cite the Family Nurture Intervention study that has demonstrated the role served by nurturing activities in infant development [1-8].

[Response] Thank you very much for the important perspective regarding the causation issue and the effects of nurturing activities on development. As we have no data to resolve the former issue, we have stated it in **Introduction** and limitation section of **Discussion**. We also have discussed the nurturing effects related to breastfeeding with these citations in **Discussion**.

The authors should also discuss possible mechanisms involved with breastfeeding that could affect neurodevelopment, especially the connection between motivation and relational health (See [9])

[Response] We have added it with the citation in **Discussion**.

[1] B. Beebe, M.M. Myers, S.H. Lee, A. Lange, J. Ewing, N. Rubinchik, H. Andrews, J. Austin, A. Hane, A.E. Margolis, M. Hofer, R.J. Ludwig, M.G. Welch, Family nurture intervention for preterm infants facilitates positive mother-infant face-to-face engagement at 4 months, Dev Psychol, 54 (2018) 2016-2031.

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[3] A.A. Hane, M.M. Myers, M.A. Hofer, R.J. Ludwig, M.S. Halperin, J. Austin, S.B. Glickstein, M.G. Welch, Family nurture intervention improves the quality of maternal caregiving in the neonatal intensive care unit: evidence from a randomized controlled trial, J Dev Behav Pediatr, 36 (2015) 188-196.

[4] S.W. Porges, M.I. Davila, G.F. Lewis, J. Kolacz, S. Okonmah-Obazee, A.A. Hane, K.Y. Kwon, R.J. Ludwig, M.M. Myers, M.G. Welch, Autonomic regulation of preterm infants is enhanced by Family Nurture Intervention, Dev Psychobiol, 61 (2019) 942-952.

[5] M.G. Welch, J.L. Barone, S.W. Porges, A.A. Hane, K.Y. Kwon, R.J. Ludwig, R.I. Stark, A.L. Surman, J. Kolacz, M.M. Myers, Family nurture intervention in the NICU increases autonomic regulation in mothers and children at 4-5 years of age: follow-up results from a randomized controlled trial, PLoS One, In Press (2020).

[6] M.G. Welch, J.L. Barone, S.W. Porges, A.A. Hane, K.Y. Kwon, R.J. Ludwig, R.I. Stark, A.L. Surman, J. Kolacz, M.M. Myers, Family nurture intervention in the NICU increases autonomic regulation in mothers and children at 4-5 years of age: Follow-up results from a randomized controlled trial, PLoS One, 15 (2020) e0236930.

[7] M.G. Welch, M.R. Firestein, J. Austin, A.A. Hane, R.I. Stark, M.A. Hofer, M. Garland, S.B. Glickstein, S.A. Brunelli, R.J. Ludwig, M.M. Myers, Family Nurture Intervention in the Neonatal Intensive Care Unit improves social-relatedness, attention, and neurodevelopment of preterm infants at 18 months in a randomized controlled trial, J Child Psychol Psychiatry, 56 (2015) 1202-1211.

[8] M.G. Welch, M.S. Halperin, J. Austin, R.I. Stark, M.A. Hofer, A.A. Hane, M.M. Myers, Depression and anxiety symptoms of mothers of preterm infants are decreased at 4 months corrected age with Family Nurture Intervention in the NICU, Arch Womens Ment Health, 19 (2016) 51-61.

[9] R.J. Ludwig, M.G. Welch, How babies learn: The autonomic socioemotional reflex, Early Hum Dev, 151 (2020) 105183.

Reviewer: 3

Prof. David Duffy, QIMR Berghofer Medical Research Institute, University of Queensland

Comments to the Author:

This seems to be an excellent resource for addressing these hypotheses.

I will largely comment on analysis.

For me, one threat to the conclusions is reverse causation, ie that subtle neurodevelopmental deficits might lead to mothers having difficulty breast feeding. Given a parental questionnaire is being used, rather than a clinical assessment, measurement might be imperfect, so developmental delay (DD) may not be reliably diagnosed. This might mean that undiagnosed DD at six months might precede

cessation of breast feeding, with the DD is only diagnosed at, say, 12 months. The effects size are small enough that this might be a consideration.

Discounting this possibility might be addressed by a longitudinal analysis of the data, since presumably repeat measurements on the same children can be identified (trajectories). For example, do delays at six months resolve by twelve months more often in children who were breast fed to twelve months?

[Response] Yes. Because simple cross tabulations would be a bit hard for readers to understand, we have added **Figure S2** and explained it in the 2nd paragraph of **Results**.

Does DD at six months predict children whose mother ceases breast feeding between six and twelve months?

[Response] No. We have added **Figure S1**.

Were participants asked to retrospectively report when they stopped breast feeding?

[Response] Yes, monthly feeding statuses were retrospectively collected at 1, 6 and 12 months old, as stated in the first part of Exposure section of **Methods**.

Rather than treating this as multiple correlated binary variables, a single length would be more informative. This would require an appropriate longitudinal linear mixed model - I would treat development scores directly as well as a dichotomized delay outcome (which would require a GLMM).

[Response] We have added multiple linear regression model for full sample (**Table 3**) and longitudinal linear mixed model for sibling pair samples (**Table 5**). In these models, exposure factor (i.e. duration of breastfeeding) and outcome (total ASQ score) were treated as continuous variables. For this purpose, especially for exclusive breastfeeding, we have changed the definition of “duration of breastfeeding”, which now represents the duration from birth. The results of a GLMM model with dichotomous outcomes did not converge.

Given that this is a well characterized cohort, I would not report odds ratios, but risk differences etc for DD.

[Response] This time, we have reported risk ratios in **Tables 2 & 4**.

*I would also like to see more results for the ***confounders*** in the sibling analysis, especially with respect to birth order. Does breast feeding of the first born predict a reduced risk of DD in the second born who was not breast fed?*

[Response] When the first born received breastfeeding, the risk of DD in the second born, who were not breastfed, were increased though statistically not significant (**Figure S3** and 3rd paragraph of **Results**).

Maternal smoking seems moderately common - how many cases are there where the mother smoked only for one pregnancy, and how does this interact with the choice to breast feed longer? These kinds of tabulations could be supplementary, but are of great interest to me in terms of causation (recalling that residual confounding from strong but imperfectly measured constructs such as social class may not be completely adjusted for by just including parental education/income).

[Response] 325 pairs were discordant for maternal smoking—mother smoked only for one pregnancy. The smoking seemed not to interact with duration of breastfeeding (**Figure S4**).

In passing, in the non-family-based analysis, consider parent-child correlation in minor developmental conditions, say a mother and infant with ADHD at various stages, that might lead to difficulties with breast feeding. A definite strength of the matched-sibling design is dealing with these possibilities. The meta-analysis of Tseng et al, 2019 for example, claims 'children with ADHD received significantly higher rate

of exclusive breastfeeding duration "under 3 months" but lower in "over 3 months" than controls' - again I am positing reverse causation as an alternative explanation here.

[Response] Unfortunately, data on developmental disorders will be provided in the future dataset. We have stated the issue of reverse causation in limitation section of **Discussion**.

VERSION 2 – REVIEW

REVIEWER	Welch, Martha G. Columbia Univ Coll Phys
REVIEW RETURNED	30-Apr-2021

GENERAL COMMENTS	Revision is sufficient, article is suitable for publication.

REVIEWER	Duffy, David QIMR Berghofer Medical Research Institute, Genetic Epidemiology, Molecular Epidemiology and Neurogenetics Laboratories
REVIEW RETURNED	05-May-2021

GENERAL COMMENTS	I feel that the authors have responded usefully to my comments. I am interested in the results for exclusive breast feeding versus

	(presumably) mixed feeding, as well as how this might fit in with the timing of introduction of complementary feeding. In Table 2, exclusive BF until 6 months seems to be N=14736 and "any" BF N=60383, so it seems to me the 3-level categorical variable "No BF6" (12967), "Mixed" (45647), "Excl BF6" (14536) might give more insight, especially if weight gain to 6 months is included in the analysis, following the comment in the Discussion that "that exclusive breastmilk may not meet the full requirements for energy and micronutrients...". I found the results of continuous ASQ score heartening. After all, dichotomizing always reduces power. The analyses of each subscale score would be useful to further sorting out causation, but I suspect the authors might do this is a later paper. Minor Points: p51 l51 "Poisson models reve[a]led" p51 l37 "breastfeeding [duration] was treated as [a] continuous variable" p54 l15 "likely" rather than "probable" p56 l12 "The current study does have several limitations". p57 l4 "although causal inference should be cautious in observational studies, both the prospective longitudinal and family-based matched analyses presented may provide a more persuasive argument.."

VERSION 2 – AUTHOR RESPONSE

Reviewer: 2

Dr. Martha G. Welch, Columbia Univ Coll Phys

Comments to the Author:

Revision is sufficient, article is suitable for publication.

[Response] Thank you very much.

Reviewer: 3

Prof. David Duffy, QIMR Berghofer Medical Research Institute, University of Queensland

Comments to the Author:

I feel that the authors have responded usefully to my comments. I am interested in the results for exclusive breast feeding versus (presumably) mixed feeding, as well as how this might fit in with the timing of introduction of complementary feeding. In Table 2, exclusive BF until 6 months seems to be N=14736 and "any" BF N=60383, so it seems to me the 3-level categorical variable "No BF6" (12967),

"Mixed" (45647), "Excl BF6" (14536) might give more insight, especially if weight gain to 6 months is included in the analysis, following the comment in the Discussion that "that exclusive breastmilk may not meet the full requirements for energy and micronutrients...".

[Response] Thank you so much for the important comments. Comparison of “No BF6 (BF discontinued by 6M: 12 967)” with “Mixed (BF continued until 6M plus formula feeding or complementary food: 60 383 – 14 735 = 45 648)” or “Excl BF6 (BF continued until 6M with neither formula nor complementary food: 14735)” might not make sense because the BF duration were different. Instead, the effects of formula feeding and complementary food that were added to breastfeeding were referenced to exclusive breastfeeding. We have added the classification and the results to ‘Exposure’ section of **Methods** and Table 2c, respectively. Concomitant ingestion of complementary food was associated with decreased developmental delay whereas concurrent formula feeding was rather associated with increased developmental delay. Unfortunately, the data of body weight at 6 months old was not collected in JECS. According to the results, we have changed the description in the 4th paragraph of **Discussion**.

I found the results of continuous ASQ score heartening. After all, dichotomizing always reduces power. The analyses of each subscale score would be useful to further sorting out causation, but I suspect the authors might do this is a later paper.

[Response] We completely agree with you. We are planning to do it using the subsequent data of this ongoing cohort.

Minor Points:

p51 l51 "Poisson models reve[a]led"

p51 l37 "breastfeeding [duration] was treated as [a] continuous variable"

p54 l15 "likely" rather than "probable"

p56 l12 "The current study does have several limitations".

p57 l4 "although causal inference should be cautious in observational

studies, both the prospective longitudinal and family-based matched

analyses presented may provide a more persuasive argument.."

[Response] We have revised the manuscript according to these proper suggestions.