PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Fish and marine fatty acids intakes, the FADS genotypes and long-term weight gain: a prospective cohort study
AUTHORS	Huang, Tao; Wang, Tiange; Heianza, Yoriko; Wiggs, Janey; Sun, Dianjianyi; Choi, Hyon-Kyoo; Chai, Jin Fang; Sim, Xueling; Khor, Chiea Chuen; Friedlander, Yechiel; Chan, Andrew T.; Curhan, Gary; Vivo, Immaculata De; van Dam, Rob Martinu.; Heng, Chew Kiat; Fuchs, Charles; Pasquale, Louis R.; Yuan, Jian-min; Hu, Frank B.; Koh, Woon Puay; Qi, Lu

VERSION 1 - REVIEW

REVIEWER	Leticia Goni University of Navarra. Spain
REVIEW RETURNED	26-Jun-2018

GENERAL COMMENTS	The paper is clear and nicely written and the results are of interest within the context of increasing the knowledge of fish and marine fatty acids, genetic variants and long-term weight gain, so in the future better recommendations can be provided. I have a couple of specific minor comments: - Line 23. Use italics for genes - Line 38. Add a reference. - Line 48. PUFA change for PUFAs - Lines 164-168. In the text is stated that there were no significant genetic association between the FADS genetic variant and BMI at endpoint, but such data are not shown in supplemental table 2. I would suggest include it. - In table 1 please specify the source of the EPA and DHA as well as total EPA and DHA. I think that this information is confusing along the manuscript. I have also doubts in table 2. I would suggest that clarify this information in the methods section.

REVIEWER	Elina Hypponen Australian Centre for Precision Health| University of South Australia Cancer Research Institute | South Australian Health & Medical Research Institute (SAHMRI) Level 8 | GPO Box 2471, Adelaide SA 5001
REVIEW RETURNED	06-Aug-2018

GENERAL COMMENTS	This is a gene-environmental interaction study, looking into effect modification genetic variations in the FADS gene on the effects by

	omega-3 fatty acid or fish intakes with respect to changes in BMI. Study includes data from four distinct cohorts, providing replicable evidence across the studies. Study is hypothesis driven with clear rational for the question set-up. Title should read 'genetic variants' as analyses were done for three SNPs. Please ensure participant inclusion criteria is clearly defined for all studies with respect to sample selection for these particular analyses. Were all analyses in this study restricted to participants with repeat information on BMI, SNP genotyping and dietary data and is this the subsample of which all total inclusion numbers reflect? Was there any variation in numbers vary between models? What did you do with missing information on covariates? Meta-analyses should be checked and corrected. a) Random effects should be used for all meta-analyses, given the heterogeneous populations. The used of fixed effects assumes all estimates are derived from the same population which clearly is not valid in this context due to ethnic and gender diversities. b) In Figure 1, weights in the meta-analysis figure appear to reflect a hypothetical contribution to a joint estimate including all dietary factors (and repeated combinations thereof) which was not presented (nor should it be). Please check that the meta-analysis is conducted appropriately, and that it does not include repeated information from a single cohort. I also suggest to delete Figure 1, as it appears to replicate information provided elsewhere in the manuscript, but if it is included please fix the weights to reflect analyses conducted. Line 228-229. Recent Cochrane review did not provide strong evidence for benefits by n-3 pufa on CVD, so suggest the authors revise the very optimistic statements in the discussion. (See PMID 30019766.) Discussion & strength/limitations: Effects by reverse causation is still a possibility as this is a study on dietary intake and BMI (weight) change from the baseline, which by default builds in the starting point (i.e. the cross sectional association). • Some tidying up of language is required, e.g. page 3 line 30 reverse causation truncated. • Please check table headings are accurate and follow a consistent style. For example,Table 2, column heading "Diets" is used for stratification by cohorts, while in similar setting in S Table 3 colum heading reads "Cohorts". Neither heading required, and suggest delete. Further in Table 2, table label suggests values within the table are for BMI change, while column heading within the table suggests Long chain n-3 Pufas and fish intakes. In supplemental table 2 (elsewhere if found), Column heading 'Adiposity' is inaccurate, when values for all rows are either BMI or BMI change. There may be other illogical labels, so please check and ensure correct notation across all tables and figures.

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

Reviewer Name: Leticia Goni

Institution and Country: University of Navarra. Spain Please state any competing interests or state ‘None declared’: None declared

The paper is clear and nicely written and the results are of interest within the context of increasing the knowledge of fish and marine fatty acids, genetic variants and long-term weight gain, so in the future better recommendations can be provided. I have a couple of specific minor comments:

Response: Thank you for your supportive comments.

- Line 23. Use italics for genes

Response: We revised it accordingly.

- Line 38. Add a reference.

Response: We added the reference.

- Line 48. PUFA change for PUFAs

Response: We changed it.

- Lines 164-168. In the text is stated that there were no significant genetic association between the FADS genetic variant and BMI at endpoint, but such data are not shown in supplemental table 2. I would suggest include it.

Response: We removed the “BMI at endpoint,”. As the results for BMI at endpoint and changes in BMI from baseline to endpoint are the same. So we did not present the results for BMI at endpoint.

- In table 1 please specify the source of the EPA and DHA as well as total EPA and DHA. I think that this information is confusing along the manuscript. I have also doubts in table 2. I would suggest that clarify this information in the methods section.

Response: Thank you for your great point. We have clarified the information on EPA and DHA.

Reviewer: 2

Reviewer Name: Elina Hypponen

Institution and Country: Australian Centre for Precision Health| University of South Australia Cancer Research Institute Please state any competing interests or state ‘None declared’: none declared

This is a gene-environmental interaction study, looking into effect modification genetic variations in the FADS gene on the effects by omega-3 fatty acid or fish intakes with respect to changes in BMI. Study includes data from four distinct cohorts, providing replicable evidence across the studies.

Study is hypothesis driven with clear rational for the question set-up.

Response: Thank you for your supportive comments.

Title should read ‘genetic variants’ as analyses were done for three SNPs.

Response: We revised it accordingly.

Please ensure participant inclusion criteria is clearly defined for all studies with respect to sample selection for these particular analyses. Were all analyses in this study restricted to participants with repeat information on BMI, SNP genotyping and dietary data and is this the subsample of which all total inclusion numbers reflect?

Response: Thank you for your thoughtful suggestions. We have included detailed information on participant inclusion criteria in method section.

Was there any variation in numbers vary between models? What did you do with missing information on covariates?

Response: We excluded participants with missing information on genetic data, exposures such as long chain n-3 PUFAs and fish intakes, baseline and endpoint BMI or major confounding factors (age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake). We have added this information in participants criteria.

Meta-analyses should be checked and corrected.

a) Random effects should be used for all meta-analyses, given the heterogeneous populations. The used of fixed effects assumes all estimates are derived from the same population which clearly is not valid in this context due to ethnic and gender diversities.

Response: When we pooled the results across cohorts, the test of heterogeneity showed that all $I2<20\%$, $P>0.05$. Therefore, we used the fixed models for each fatty acid or fish intake. We have added these statistical methods in methods and Figure 1 footnote.

Ref: Quantifying heterogeneity in a meta-analysis. Higgins JP, Thompson SG Stat Med. 2002 Jun 15; 21(11):1539-58.

b) In Figure 1, weights in the meta-analysis figure appear to reflect a hypothetical contribution to a joint estimate including all dietary factors (and repeated combinations thereof) which was not presented (nor should it be). Please check that the meta-analysis is conducted appropriately, and that it does not include repeated information from a single cohort. I also suggest to delete Figure 1, as it appears to replicate information provided elsewhere in the manuscript, but if it is included please fix the weights to reflect analyses conducted.

Response: Thank you for your thoughtful comments and suggestions, which are very helpful. We have revised the weights in figure 1.

Line 228-229. Recent Cochrane review did not provide strong evidence for benefits by n-3 pufa on CVD, so suggest the authors revise the very optimistic statements in the discussion. (See PMID 30019766.)

Response: Thanks for your suggestions, which are very helpful. We have removed the statement “Compelling evidence has shown that fish- and long chain n-3 PUFAs-rich diet are beneficial on improvement of cardiometabolic health”.

Discussion & strength/limitations: Effects by reverse causation is still a possibility as this is a study on dietary intake and BMI (weight) change from the baseline, which by default builds in the starting point (i.e. the cross sectional association).

Response: Thank you for your thoughtful comments. We agreed with you, and revised it accordingly.

“Although we prospectively analyzed the data, we cannot exclude the possibility of reverse causality as this is a study on dietary intake and BMI or weight change from the baseline, which by default builds in the starting point (i.e. the cross sectional association).”

- Some tidying up of language is required, e.g. page 3 line 30 reverse causation truncated.

Response: We have double checked the language throughout the manuscript and revised it accordingly.

- Please check table headings are accurate and follow a consistent style. For example, Table 2, column heading “Diets” is used for stratification by cohorts, while in similar setting in S Table 3 colum heading reads “Cohorts”. Neither heading required, and suggest delete.

Response: We delete “diets” in Table 2 and “Cohorts” in S Table 3.

Further in Table 2, table label suggests values within the table are for BMI change, while column heading within the table suggests Long chain n-3 Pufas and fish intakes.

Response: We agreed that the table 2 is confusing. Therefore, we added footnote that “Data are means ± SE for long term changes in BMI.” And also clarified the column heading.

In supplemental table 2 (elsewhere if found), Column heading ‘Adiposity’ is inaccurate, when values for all rows are either BMI or BMI change. There may be other illogical labels, so please check and ensure correct notation across all tables and figures.

Response: We changed the “Adiposity” to “Outcomes”.

VERSION 2 – REVIEW

REVIEWER	Prof. Anthony Atkinson London School of Economics, London, UK
REVIEW RETURNED	19-Jan-2019

GENERAL COMMENTS	Report for the authors on ‘BMJ Open’ submission bmjopen-2018-022877.R1 “Fish and marine fatty acids interacted with genetic variants of FADS gene in influencing long-term weight gain” by Huang and many othersSchenkendorf and 4 others I was asked especially to look at statistical matters, on which I do have a few comments. I will follow these with some comments on improving the ease of reading. I expect these are not all and I suggest your co-author Janey Wiggs (whom I do not know but is presumably anglophone) read through the paper carefully with my comments to hand. l.147. ‘general linear models’. This is such a general term that I’m never quite sure what is meant (apart from the confusion with generalized linear models). I think of them as being regression models, with the variances of the observations being unequal, so you have a covariance matrix Σ. Wikipedia inclines towards multivariate responses. But I think what you have is multiple linear regression sometimes with a single interaction term. This is, of course, a special case of the GLM, but is a more informative specification of your model. (This occurs many times).

	l.157. This is the first time I have seen your paper. Perhaps the yellow highlighted passages are your revisions. I asked BMJ, but had no meaningful response. Anyway, there are many meta-analytic proceedings. Could you give a reference to what you used? l.159. 'Hardy-Weinberg'. I expect you tested this because of a box you had to fill in on a form between the two versions of your paper. But what were the results of the test, what the conclusions? l.188 (also l.16). 'three tertiles'. These are presumably the three categories in Table 2. Are these just observed frequencies in the categories? If they are tertiles you must have taken a continuous variable, found the two tertiles giving 33% and 67% of the population and then what? Taken the medians of these three groups? But that wouldn't give these neat boundaries. Figs 3 and 4. It is more informative to plot fitted lines (as you have) and observed, rather than predicted, values. In Fig.3 I would, if I were you, worry about the potentially strong effect of the few observations in the right-hand third of the plot - the effect of leverage points. Table captions. All four have something about fixed effects meta-analysis (if $P \geq 0.05$). So this is when the data are homogeneous. As I mentioned above, what meta-analysis. Why not, in this case, just pool the data? What do you do if $P < 0.05$? Somewhere you mention weighting inversely by variances. That seems sensible in the case of heterogeneity. Now for some smaller points. Phrases in 'inverted commas' are suggested improvements. l.28. US police forces use the word "Caucasian" to describe "people with European ancestry". I don't know if it is a normal epidemiological term, but you don't need to say both (several times). l.138. Two groups have the same coding. Perhaps this is a typo. And some small points. l.6. Not clear. 'in BMI and body weight using four prospective cohort studies'. (4 not 3 and structure of sentence). l.9. What's 'replicated' for? I thought all 4 data sets were considered equally. l.33. This sentence should all be in the plural. 'Diets', 'acids, especially ...), have'. l.34. 'cardiometabolic health. However' l.39. 'genes'. l.51. Here is 'replicated'. From what I read, I concluded you found this in all four datasets. You could have replicated some earlier findings, for example, but I don't find that structure here. l.52. '(WHI, US)'. l.76. 'was also approved'. l.85. 'consumption and covariate'. l.94. 'of the Singapore'. l.109. Omit first 'both'. ll.125/6. Should this be 'Alternative healthy? Omit 'respectively' (hardly ever needed.) l.143. 'Neither patients nor public were involved'. l.168. A strange tense. '4 cohorts is'. l.170. 'in the three US cohorts ($P > 0.05$). However, we' l.175 'variously'. l.183. 'samples' not 'populations'. l.232. 'have generated'. l.234. 'lend support'.

	l.242. ‘of the T allele’. l.245. ‘differently to weight’. l.248. ‘a diet low’. l.257. ‘unclear. However,’ l.264. What about ‘Further,’ ? l.265. ‘exposed to extremely low temperatures’ l.268/9. ‘may not have much benefit for Europeans’. l.281. ‘inevitable. Despite this, the’. l.293. ‘Our data provide’.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 3

Reviewer Name: Prof. Anthony Atkinson

Institution and Country: London School of Economics, London, UK Please state any competing interests or state ‘None declared’: None declared

Report for the authors on ‘BMJ Open’ submission bmjopen-2018-022877.R1 “Fish and marine fatty acids interacted with genetic variants of FADS gene in influencing long-term weight gain” by Huang and many othersSchenkendorf and 4 others

I was asked especially to look at statistical matters, on which I do have a few comments. I will follow these with some comments on improving the ease of reading. I expect these are not all and I suggest your co-author Janey Wiggs (whom I do not know but is presumably anglophone) read through the paper carefully with my comments to hand.

Response: We asked our co-authors to read through the paper carefully and revised it accordingly.

l.147. ‘general linear models’. This is such a general term that I’m never quite sure what is meant (apart from the confusion with generalized linear models). I think of them as being regression models, with the variances of the observations being unequal, so you have a covariance matrix Σ. Wikipedia inclines towards multivariate responses. But I think what you have is multiple linear regression sometimes with a single interaction term. This is, of course, a special case of the GLM, but is a more informative specification of your model. (This occurs many times).

Response: Thank you for your thoughtful comments. We used multiple linear regression models with a single interaction term. We revised it throughout the manuscript.

l.157. This is the first time I have seen your paper. Perhaps the yellow highlighted passages are your revisions. I asked BMJ, but had no meaningful response. Anyway, there are many meta-analytic proceedings. Could you give a reference to what you used?

Response: Yes, results across cohorts were pooled with inverse variance weighted meta-analyses by fixed effects models (if $P \geq 0.05$ for heterogeneity between studies) or random effects models (if $P < 0.05$ for heterogeneity between studies). We have added the reference on page 10.

Ref: Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Statistics in medicine 2002;21(11):1539-58

l.159. ‘Hardy-Weinberg’. I expect you tested this because of a box you had to fill in on a form between the two versions of your paper. But what were the results of the test, what the conclusions?

Response: In this study, chi-square test showed that the FADS rs174570 is in Hardy-Weinberg equilibrium. We have added this result to Results section on page 10.

l.188 (also l.16). ‘three tertiles’. These are presumably the three categories in Table 2. Are these just observed frequencies in the categories? If they are tertiles you must have taken a continuous variable, found the two tertiles giving 33% and 67% of the population and then what? Taken the medians of these three groups? But that wouldn’t give these neat boundaries.

Response: Yes, the three tertiles are the three categories for n-3 PUFA. We take it as a continuous variable after assign the medians of these three groups. This is wide used in nutritional epidemiological study to test the p value for trends. We have added the method in statistical analyses section as “Linear trend across categories of long chain n-3 PUFAs and fish intakes was quantified with a Wald test for linear trend by assigning the median value to each category and modeling it as a continuous variable.”

Figs 3 and 4. It is more informative to plot fitted lines (as you have) and observed, rather than predicted, values. In Fig.3 I would, if I were you, worry about the potentially strong effect of the few observations in the right-hand third of the plot - the effect of leverage points.

Response: Thank you for your thoughtful comments and suggestions. To understand how fish or long chain n-3 PUFA intakes predict the weight changes according to genotypes, we used predicted values rather than observed values.

We acknowledge that there is possibility that the few observations in the right-hand third of the plot in Figure 3 may have strong effect on results. Therefore, in abstract and discussion, we added that “Further investigation is needed to confirm our findings in other cohorts.”

Table captions. All four have something about fixed effects meta-analysis (if $P \geq 0.05$). So this is when the data are homogeneous. As I mentioned above, what meta-analysis. Why not, in this case, just pool the data? What do you do if $P < 0.05$? Somewhere you mention weighting inversely by variances. That seems sensible in the case of heterogeneity.

Now for some smaller points. Phrases in ‘inverted commas’ are suggested improvements.

Response: As the test for heterogeneity between studies was not significant ($P>0.05$), the results across the four cohorts were pooled with inverse variance weighted meta-analyses by fixed effects models. We have revised the meta-analysis methods as “Results across cohorts were pooled with inverse variance weighted meta-analyses by fixed effects models ($P \geq 0.05$ for heterogeneity between studies)” on page 9.

We also revised the table captions accordingly.

l.28. US police forces use the word “Caucasian” to describe “people with European ancestry”. I don’t know if it is a normal epidemiological term, but you don’t need to say both (several times).

Response: We have changed the words “Caucasians with European ancestry” to “Caucasians”.

l.138. Two groups have the same coding. Perhaps this is a typo. And some small points.

l.6. Not clear. ‘in BMI and body weight using four prospective cohort studies’. (4 not 3 and structure of sentence).

Response: We have revised the sentence accordingly.

l.9. What’s ‘replicated’ for? I thought all 4 data sets were considered equally.

Response: In the present study, NHS and HPFS studies are discovery cohorts. We replicated the observed results in WHI and SCHS cohorts which are replication cohorts.

l.33. This sentence should all be in the plural. ‘Diets’, ‘acids, especially...), have’.

l.34. ‘cardiometabolic health. However’

l.39. ‘genes’.

Response: We have revised the manuscript according to above suggestions.

l.51. Here is ‘replicated’. From what I read, I concluded you found this in all four datasets. You could have replicated some earlier findings, for example, but I don’t find that structure here.

Response: In the present study, NHS and HPFS studies are discovery cohorts. We replicated the observed results in WHI and SCHS cohorts which are replication cohorts.

l.52. ‘(WHI, US)’.

l.76. ‘was also approved’.

l.85. ‘consumption and covariate’.

l.94. ‘of the Singapore’.

l.109. Omit first ‘both’.

ll.125/6. Should this be ‘Alternative healthy? Omit ‘respectively’ (hardly ever needed.)

l.143. ‘Neither patients nor public were involved’.

l.168. A strange tense. ‘4 cohorts is’.

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l.265. ‘exposed to extremely low temperatures’

l.268/9. ‘may not have much benefit for Europeans’.

l.281. ‘inevitable. Despite this, the’.

l.293. ‘Our data provide’.

Response: We have revised the manuscript according to above suggestions.

VERSION 3 – REVIEW

REVIEWER	Prof. Anthony C. Atkinson Dept of Statistics, London School of Economics, UK
REVIEW RETURNED	15-Mar-2019

GENERAL COMMENTS	Report for the authors on revised ‘BMJ Open’ submission bmjopen-2018-022877.R2 “Fish and marine fatty acids intakes modified the genetic effects of the FADS gene on long-term weight gain: a gene-diet interaction analysis” by Huang and many others Thank you for the careful revision of your paper. I find the English is now serviceable and unambiguous. My only query remains Figures 3 and 4. What does each dot represent? The only assumption I can make is that these x values were found by pooling. You don’t mention this around l.159. I am not clear about the response. In the graph we have predicted 10 year change in BMI. But in the description around l.127 you have some ten year changes and some 6 year changes. These are in different cohorts, so model fitting within cohort would not be a problem. What did you do then? Does your meta-analysis combine these estimates; perhaps you have included length of observation, or you may have assumed they were annual changes (that is after division of the responses by 6 or 10). If this is what is happening, how do your statements about standard errors allow for the fact that some ten year predictions are based on 6 year observations? I think it would be interesting to plot the data (scaled up for the 6 year observations) in the figures as well as the fitted lines, with different symbols for the 6 and 10 year studies. The responses were, I suppose, pooled just like the x values. So there are not thousands of them, especially for Figure 4. l.169. Is this Wald test standard? Perhaps you could give a reference. On the face of it it seems a strange choice.

VERSION 3 – AUTHOR RESPONSE

Report for the authors on revised ‘BMJ Open’ submission bmjopen-2018-022877.R2 “Fish and marine fatty acids intakes modified the genetic effects of the FADS gene on long-term weight gain: a gene-diet interaction analysis” by Huang and many others

Thank you for the careful revision of your paper. I find the English is now serviceable and unambiguous.

Response: Thank you for your supportive comments.

My only query remains Figures 3 and 4. What does each dot represent? The only assumption I can make is that these x values were found by pooling. You don’t mention this around l.159. I am not clear

about the response. In the graph we have predicted 10 year change in BMI. But in the description around l.127 you have some ten year changes and some 6 year changes. These are in different cohorts, so model fitting within cohort would not be a problem. What did you do then? Does your meta-analysis combine these estimates; perhaps you have included length of observation, or you may have assumed they were annual changes (that is after division of the responses by 6 or 10). If this is what is happening, how do your statements about standard errors allow for the fact that some ten year predictions are based on 6 year observations? I think it would be interesting to plot the data (scaled up for the 6 year observations) in the figures as well as the fitted lines, with different symbols for the 6 and 10 year studies. The responses were, I suppose, pooled just like the x values. So there are not thousands of them, especially for Figure 4.

Response: Thank you for your great comments. The individual participant data from the NHS and HPFS cohorts were pooled to generate the predicted 10-year changes in body weight according to the FADS genotypes. We have added this in statistical methods. The way of plotting predicted outcome according to genotypes has been widely used in examining gene-environment interaction.

l.169. Is this Wald test standard? Perhaps you could give a reference.

On the face of it seems a strange choice.

Response: The Wald test has been widely used to quantify the linear trend by assigning the median value to each category and modeling it as a continuous variable. We have added the reference in Line 167.