PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The Association between Age, Gender, Menopausal Status, and Small Dense Low-Density Lipoprotein Cholesterol; A Cross-Sectional Study
AUTHORS	Izumida, Toshihide; Nakamura, Yosikazu; Sato, Yukihiro; Ishikawa, Shizukiyo

VERSION 1 – REVIEW

REVIEWER	Danny Liew Monash University, Australia.
REVIEW RETURNED	19-Jul-2020

GENERAL COMMENTS	Izumida and colleagues undertook a cross-sectional study of small dense low-density lipoprotein cholesterol (sdLDL-C) and low-density lipoprotein cholesterol (LDL-C) fractions in a rural-dwelling Japanese population. The methods were well described. I have 2 major comments: 1. It seems that the study should primarily be a descriptive one, with the aim of profiling sdLDL-C (a relatively novel cardiovascular risk factor) and LDL-C fractions in the target population, including stratified by age-groups, gender and menopausal status. Instead, the study adopts an 'analytical' angle, focusing on addressing the hypothesis that there are associations between demographic factors and the lipid parameters. It does not seem that this hypothesis needs addressing, or at least the authors need to better establish the rationale that it does. If the authors change the intent of their study to a descriptive one, then it does not need regression analyses. Simple comparative analyses will suffice. 2. A major limitation of the study is selection bias arising from potential non-representativeness of the study population, which was rural dwelling. The authors did not acknowledge this. Nor did they acknowledge the possibilities for information bias and data misclassification (eg, error in measurement of the lipid parameters).

REVIEWER	Dr Peter Penson Liverpool John Moores University, UK I own four four shares in AstraZeneca PLC and havereceived honoraria and/or travel reimbursement for events sponsored by AKCEA, Amgen, AMRYT, Link Medical, Napp, Sanofi;

REVIEW RETURNED	22-Jul-2020

GENERAL COMMENTS	I enjoyed reading your interesting manuscript describing your cross-sectional study of sdLDL. Please consider the following comments and suggestions: -Throughout the manuscript, you use language that implies causality (e.g. 'the effect of X on Y'). A cross-sectional observational study can only demonstrate associations (not causality). The language should be updated to reflect this fact. -The introduction discusses curvilinear relationships between variables, but your analysis is essentially linear. Did you consider using nonlinear regression, splines etc.? -Whilst you clearly state that this is a cross-sectional study in the abstract, this doesn't come across clearly in the first paragraph of the methods. It would be helpful to re-iterate this fact -In your methods section, you state that you excluded patients taking lipid-lowering therapy. You should extensively discuss how this affects your study population and the external validity of your work (have you ended up by default with a relatively 'low-risk' population)? sdLDL-C/LDL-C ratio is one of the key endpoints in your study. I think further explanation is needed as to why this measurement is important. Has it been validated in risk prediction? Does it matter that it tells us nothing about the absolute concentration of sdLDL-C? Your statistical analysis section (L164) introduces the 'three groups' these are not clearly articulated here or earlier in the paper - it would be helpful if they were. In your statistical analysis section L171-173 you discuss agreement between estimated and measured values, but it is not clear how these estimates have been derived. In the text of your results section you state various beta values, however, as presented, it is hard to get an appreciation of the 'effect size' (how much one variable changes with another) without looking in tables to see what measurement all the units are variables are measured in - can you make this clearer for the reader? -Given the importance to the research question, I think the description of LDL-measurement 'by direct methods' is insufficiently detailed. The assay should be more fully described. -I agree with you that measuring sdLDL-C is an advance over simply measuring (or calculating) LDL-C. Nevertheless, I think it is a weakness (which should be acknowledged) that Lp(a) was not quantified in this study.

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1
Reviewer Name
Danny Liew

Institution and Country
Monash University, Australia.

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I have 2 major comments:
1. It seems that the study should primarily be a descriptive one, with the aim of profiling sdLDL-C (a relatively novel cardiovascular risk factor) and LDL-C fractions in the target population, including stratified by age-groups, gender and menopausal status. Instead, the study adopts an 'analytical' angle, focusing on addressing the hypothesis that there are associations between demographic factors and the lipid parameters. It does not seem that this hypothesis needs addressing, or at least the authors need to better establish the rationale that it does.

If the authors change the intent of their study to a descriptive one, then it does not need regression analyses. Simple comparative analyses will suffice.

Dear. Danny Liew.

Thank you for your review and comment.
Our study adopted an analytical angle, focusing on addressing the hypothesis that there are associations between demographic factors and the lipid parameters. We rephrased the sentences and modified our paper.
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2. A major limitation of the study is selection bias arising from potential non-representativeness of the study population, which was rural dwelling. The authors did not acknowledge this. Nor did they acknowledge the possibilities for information bias and data misclassification (eg, error in measurement of the lipid parameters).

We have added the sentence below to limitations.
Fourth, there might be several biases. Selection bias might come from potential non-representativeness of the study population, which was rural dwelling. There might be information bias and data misclassification due to error in measurement of the lipid parameters.
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Reviewer: 2
Reviewer Name
Dr Peter Penson

Institution and Country
Liverpool John Moores University, UK

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-Throughout the manuscript, you use language that implies causality (e.g. 'the effect of X on Y'). A cross-sectional observational study can only demonstrate associations (not causality). The language should be updated to reflect this fact.

Dear Peter Penson.
Thank you for your review and comment. We stopped using language that implies causality. We rephrased the sentences and modified our paper.

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-The introduction discusses curvilinear relationships between variables, but your analysis is essentially linear. Did you consider using nonlinear regression, splines etc.?

When we constructed the protocol of our study, whether we should use nonlinear regression in statistic method was discussed. After all, we decided to use linear analysis using 5-year age groups rather than nonlinear regression, because the methods of the previous reports regarding the association between lipid markers and the demographic factors including age were linear analysis using 5-year age groups and several studies showed the linear association between specific age groups and lipid parameters We modified our paper, considering the above mentioned.
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-Whilst you clearly state that this is a cross-sectional study in the abstract, this doesn't come across clearly in the first paragraph of the methods. It would be helpful to re-iterate this fact

We modified the methods of our paper as the sentence below.
METHODS
Population
The present cross-sectional study was conducted as part of the Jichi Medical School (JMS)-II Cohort Study, a population-based cohort study of the risk factors of atherosclerosis and CVD in the Japanese general population.
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-In your methods section, you state that you excluded patients taking lipid-lowering therapy. You should extensively discuss how this affects your study population and the external validity of your work (have you ended up by default with a relatively 'low-risk' population)?

We reanalyzed all participants including patients taking lipid-lowering therapy.
As shown in the reanalyzed figure below, the results regarding the association between demographic factors and sdLDL-C and sdLDL-C/LDL-C ratio remained the same. However, sdLDL-C/LDL-C ratio in men including patients taking lipid-lowering therapy was higher than in men excluding these patients (0.45 vs 0.35). In all participants, 50-year old standardized sdLDL-C levels and sdLDL-C/LDL-C ratio in men, premenopausal women, and postmenopausal women were 28.4 mg / dL (95 % CI; 28.2-28.6 mg / dL), 22.2 mg / dL (95 % CI; 22.0-22.4 mg / dL), and 26.4 mg / dL (95 % CI; 25.9-27.0 mg/dL) and 0.38 (95 % CI; 0.37-0.38), 0.18 (95 % CI; 0.18-0.19), and 0.18 (95 % CI; 0.18-0.19), respectively. Our assessment was limited in terms of the differences between the results in all participants and these in participants excluding patients taking lipid-lowering medications, because data regarding type and dose of medications for dyslipidemia were not available and the medication might affect the levels of sdLDL-C and sdLDL-C/LDL-C ratio. We need to validate the association in a relatively high-risk population, such as patients taking lipid-lowering therapy in the future.

We modified our disccusion section of our paper as the sentence below.

As shown in the supplementary figure 6 and 7, the results regarding the association between demographic factors and sdLDL-C and sdLDL-C/LDL-C ratio remained the same in 6,282 participants including patients taking lipid-lowering therapy.
SdLDL-C/LDL-C ratio in men including patients taking lipid-lowering therapy was higher than in men excluding these patients (0.45 vs 0.35). Our assessment was limited in terms of this difference, because data regarding type and dose of medications for dyslipidemia were not available. We need to validate the association in patients taking lipid-lowering therapy in another cohort.

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sdLDL-C/LDL-C ratio is one of the key endpoints in your study. I think further explanation is needed as to why this measurement is important. Has it been validated in risk prediction? Does it matter that it tells us nothing about the absolute concentration of sdLDL-C?

We added the below sentence to intoroduction.
The sdLDL-C / LDL-C ratio, reflecting the ability to generate sdLDL-C from LDL-C, might increase by the high activity of hepatic lipase, which might be associated with higher risk of CVD. Current studies suggest that the sdLDL-C or sdLDL-C/LDL-C ratio might be the better factors for the prediction of CVD than total cholesterol (TC) or LDL-C in the general population or patients with CVD.
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Your statistical analysis section (L164) introduces the 'three groups' these are not clearly articulated here or earlier in the paper - it would be helpful if they were.

We added the below sentence to the statistical analysis of our paper.
The participants were divided into three groups (men, premenopausal women, and postmenopausal women) according to gender and menopausal status.
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In your statistical analysis section L171-173 you discuss agreement between estimated and measured values, but it is not clear how these estimates have been derived.

We added the below sentence to our paper.
Considering the beta value of age, body mass index, fasting glucose, and smoking and drinking status, we calculated the estimated sdLDL-C and sdLDL-C/LDL-C ratio.
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In the text of your results section you state various beta values, however, as presented, it is hard to get an appreciation of the 'effect size' (how much one variable changes with another) without looking in tables to see what measurement all the units are variables are measured in - can you make this clearer for the reader?

We modified the result section of our paper.

Among premenopausal women, postmenopausal women ≤ 64 years, and postmenopausal women 65 ≥ years, age was positively, positively, and negatively associated with LNsdLDL-C levels. But the association between LNsdLDL-C and age was not significantly associated with men ≤ 54 years.

In women, age in premenopausal women, postmenopausal women ≤ 69 years was positively associated with sdLDL-C/LDL-C ratio, whereas age in postmenopausal women 70 ≥ years was not significantly associated sdLDL-C/LDL-C ratio.
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-Given the importance to the research question, I think the description of LDL-measurement 'by direct methods' is insufficiently detailed. The assay should be more fully described.

We added the data and modified our paper.
LDL-C and high-density lipoprotein cholesterol (HDL-C) were measured by direct methods using a commercial kit (Cholestest from Sekisui Medical, Tokyo, Japan).
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-I agree with you that measuring sdLDL-C is an advance over simply measuring (or calculating) LDL-C. Nevertheless, I think it is a weakness (which should be acknowledged) that Lp(a) was not quantified in this study.

Thank you for your comment. Lp(a) was a significant risk factor for cardiovascular disorders and to be in the spotlight due to a novel therapy using antisense oligonucleotides. Considering that the values of Lp(a) may vary between laboratories as measurement and target levels have not been standardized, the association between the demographic factors and Lp(a) should be discussed in further study. We added the sentence below to the limitation section of our paper.

Finally, our study could not evaluate the association between the demographic factors and other lipid markers, such as Lp(a) and oxidized LDL-C. Lp(a) was a significant risk factor for cardiovascular disorders and to be in the spotlight due to a novel therapy using antisense oligonucleotides. These lipid markers should be discussed in further study.

VERSION 2 – REVIEW

REVIEWER	Peter Penson Liverpool John Moores University, UK I own four shares in AstraZeneca PLC I have received honoraria and/or travel reimbursement for events sponsored by AKCEA, Amgen, AMRYT, Link Medical, Mylan, Napp, Sanofi.
REVIEW RETURNED	21-Oct-2020

GENERAL COMMENTS	Thank you for addressing my comments.