Peer Review File

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Reviewer A

Kim et al. have analyzed serum or plasma levels of the transmembrane protein LRIG2 in patients with newly diagnosed type 2 diabetes, prediabetes, or normal glucose tolerance. The results showed that the average LRIG2 level was increased in the type 2 diabetes group. To the best of the reviewer's knowledge, this is the first report describing plasma levels of LRIG2 in human subjects. The results are potentially interesting, however, the clinical utility of LRIG2 as a biomarker for type 2 diabetes remains doubtful.

Specific comments:

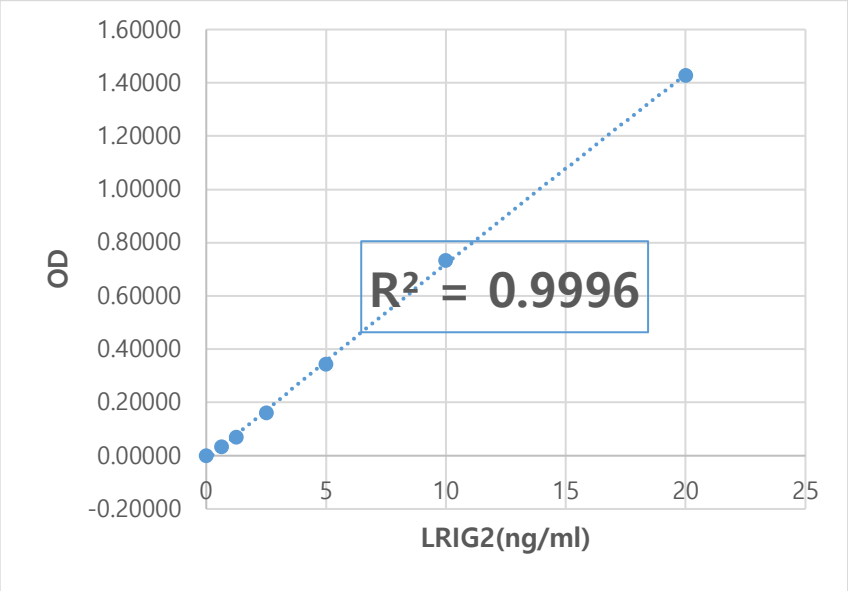
1. Was it serum or plasma that was analyzed? The information provided in the Abstract and Methods sections are discordant in this regard.

In present study, we measured serum soluble LRIG2 and clinical parameters. The serum samples were obtained by centrifuging the blood. We added this in method section (biochemical data).

2. How was the LRIG2 ELISA valdiated? The study is based on LRIG2 measurements using only a single method, i.e., a commercially available ELISA kit. Hence, the validity of this ELISA is critically important for the current study. However, the assay does not seem to have been published previously, nor is any other validation-information provided.

We agree with your opinion. In present study, the fasting serum sLRIG2 level was measured using a quantitative sandwich enzyme immunoassay technique with an enzyme-linked immunosorbent assay (ELISA) kit (MyBioSource, San Diego, CA, USA; catalog number MBS9337066). To our knowledge, this is the first study to measure serum soluble LRIG2 levels using this commercial ELISA kit. About 3 commercial LRIG2 ELISA kits are available today. We measured soluble LRIG2 using ELISA kit manufactured by MyBioSource because MyBioSource offers highly validated ELISA kit. According to MyBioSource's information, this LRIG2 ELISA kit has CV values less than 20% across the standard curve for both intra-

and inter-assay precision (intra-assay CV <15%, inter-assay CV <15%). We added this information in "methods" section. In addition, we obtained reliable standard curves in order to obtain valid and accurate results (R^2 0.9996).



3. In the Introduction, the authors review the role of LRIG proteins in cancer and in the regulation of receptor tyrosine kinases. However, in the Discussion the authors mention a recent report showing that LRIG1 is a determinant of BMI, risk of type 2 diabetes, and BMP signaling. Given the subject of the manuscript, it would be appropriate to introduce this study already in the Introduction.

Thank you for your comment. We introduce the report briefly in the last paragraph of "introduction" section.

4. In diabetes, many phsyiological functions are affected, including various vessel functions. Accordingly, many different proteins have been reported to be increased in plasma of diabetic patients; for example, the authors have themselves shown that sEGFR is increased among patients with type 2 diabetes. Hence, it would be appropriate to discuss the current sLRIG2 results in relation to these previous data.

Thank you for your comment. As type 2 diabetes is a growing worldwide health problem, there is a need to develop effective diagnostic biomarker for diabetes. Numbers of molecules, such as high-sensitivity C-reactive protein (hsCRP), fibroblast growth factor 21 (FGF21), adiponectin and microRNA, have been proposed to be potential biomarker. However, these biomarkers have limitations in the early detection of diabetes. To investigate the usefulness of

serum soluble LRIG2 as biomarker for the early diagnosis of type 2 diabetes, we enrolled only subject with drug-naïve, newly diagnosed type 2 diabetes.

References

1. Clin Endocrinol (Oxf). 2017 Jan;86(1):37-43
2. Mol Med Rep. 2015 Nov;12(5):7485-90.
3. Diabetes Care. 2012;35(12):2540–2547.
4. Cardiovasc Diabetol . 2014 Feb 4;13:34

We add the above in the first paragraph of "discussion section" and related references.

5. The authors state that sLRIG2 might be a diagnostic biomarker for type 2 diabetes and that they have demonstrated its 'usefulness'. These claims should be thouroughly discussed. What was the sensitivity and specificity of sLRIG2 as a biomarker for type 2 diabetes? How could sLRIG2 assays be useful? In what way is sLRIG2 superior to other biomarkers, or how may they complement each other, etc.?

Thank you for your comments. To evaluate the sensitivity and specificity of serum sLRIG2 in the diagnosis of type 2 diabetes, the area under the receiver operating characteristic (ROC) curve was performed. The area under the ROC curve was 0.768 and the best cut-off value for serum sLRIG2 was 15.1 ng/mL (sensitivity 66.25%, specificity 74.37%). We have added this results in the "results" section.

We reviewed previous papers that had identified novel serum or plasma biomarker for type 2 diabetes. Previous reports suggested that ROC AUC of most serum or plasma biomarkers were 0.6-0.8. We confirmed that serum sLRIG2 has no inferior diagnostic accuracy compared to other biomarkers for type 2 diabetes. Further research is needed to demonstrate whether combination use of sLRIG2 and sEGFR as diagnostic biomarker improve their diagnostic value.

Reviewer B

The present study investigated whether serum soluble leucine-rich repeats and immunoglobulin like domains 2 (sLRIG2) was useful as a diagnostic biomarker of type 2 diabetes.
The authors concluded that since the serum sLRIG2 levels correlated with glucose parameters, sLRIG2 might be a novel diagnostic biomarker for type 2 diabetes.

Comments

1. How was the definition Prediabetes? Did you mean IFG or IGT? Please describe the definition of Prediabetes in the Method section.

Prediabetes was defined as fasting plasma glucose 100 to 125 mg/dL or 2-hour postprandial glucose 140 to 199 mg/dL or hemoglobin A1C (HbA1c) 5.7 to 6.4% according to the ADA criteria. We add this criteria in the "methods" section.

2. Did you look at changes in sLTIG2 levels during a 75-g oral glucose load?

Thank you for your opinion. Unfortunately, we couldn't determine serum sLRIG2 levels in 2-hour postprandial samples.

3. Similarly, did you compare sLTRG2 levels in a fasting with a postprandial state?

As we mentioned above, we couldn't confirm whether acute glucose fluctuation affect serum sLRIG2 levels because we measured serum sLRIG2 levels only in fasting samples.
We add this study limitation in "discussion" section.

4. The authors should undertake ROC curve analysis of sLRIG2 for diagnosis of type 2 diabetes.

Thank you for your comments. The area under the ROC curve was 0.768 and the best cut-off value for serum sLRIG2 was 15.1 ng/mL (sensitivity 66.25%, specificity 74.37%). We have added the ROC curve of sLRIG2 for diagnosis of T2DM in the "results" section.