



SDI Review Form 1.6

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Manuscript Number:	Ms_BJMMR_27076
Title of the Manuscript:	Lipoprotein (a) particles characterization by Dynamic Light Scattering
Type of the Article	Original Research Article

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	<p>In the present study, authors attempted to characterize plasma-derived Lp(a) particles from homozygous individuals using Dynamic Light Scattering (DLS). DLS is a method that can estimate the mean nanoparticle size in fluids by measuring the intensity fluctuation of scattered light. The estimation of particle sizes is possible by measuring the light-scattering intensity of the particles with the random movements, or Brownian motion. This method is rapid and easy to be used. Previous studies have reported the use of DLS for measuring the sizes of lipoproteins, such as human LDL and chylomicrons from human lymph. However, the feasibility of using DLS to differentiate lipoprotein subclasses has been mainly investigated by using other methods such as NMR. Finding may provide some useful information that can be used for future studies exploring the role of some specific Lp(a) subclasses in CVD inflammatory process.</p> <p>However, the method is not fully validated to reach final conclusion. One possible major issue with DSL method is when a mixture of differently sized particles is measured; the average size</p>	<p>The Reviewer's observations about the need to validate the technique has been reported in the Conclusion's section, underlining also the need to take advantage of different methods run in parallel on homogeneous samples. Regarding the single issues raised:</p> <p>1/ These suggestions are very welcome and, along with those proposed in point 6 below, were included in the Discussion section, created anew according to the suggestion of the second Reviewer. One problematic point, as also mentioned in point 6, is accessibility to enough volume of plasma samples to cover all the experiments, which is a strong limitation. Particularly, according to existing regulation for plasma donations and for ethical reasons, CVD patients are unlikely to be considered potential donors for these kinds of experiments. All the available techniques, in fact, are not micromethods, but require significant amounts of plasma, especially to allow for replicates of</p>



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	<p>estimated by the DLS measurement is susceptible to the change in large particles. Therefore, it is recommended to perform the DLS measurement with more than one separation technique to eliminate unnecessary contaminant particles in biological specimens. Authors raised several issues with regards to potential artifacts that could be generated from Lp(a) degradation or/and to aggregation of particles in concentrated samples.</p> <p>1/ Authors may need to compare several isolation and purification of Lp(a) particles (gel chromatography/ultracentrifugation) using some internal negative/positive controls (sera from other CVD?).</p> <p>2/ Blot/westerns are cited but not shown. Need to be provided to show differences among sera of homozygous patients.</p> <p>3/ Reference for ELISA need to be provided. What standard is used? Please indicate assay precision/coefficients CVs, etc.</p> <p>4/ Lp(a) samples were concentrated to 1 mg/ml concentration. How? What is the nature and composition of buffer?</p> <p>5/ Samples were concentrated to 1 mg/ml. How? How did you control for particles aggregation?</p> <p>6/ In order achieve better accuracy and precise measurement, the technique used here should be coupled with a more convenient isolation technique for homogenous Lp(a) subpopulations.</p>	<p>experiments.</p> <p>For this reason, as also mentioned in the reply to point 6 below, we are planning to produce different isoforms of Lp(a) and apo(a) in recombinant form.</p> <p>2/ The western blotting figure was introduced as Figure 1 of the first paragraph of the Results section (<i>Apo(a) molecular weight determination</i> paragraph).</p> <p>3/ A new description of the ELISA assay was supplied in the Material and Methods section (<i>Determination of Lp(a) concentration</i> paragraph), including standards and precision CV coefficients.</p> <p>4/ The concentration procedure used for the samples and the buffer compositions were introduced in the Material and methods section (<i>Size measurements</i> paragraph).</p> <p>5/ See above (point 4) for the concentration procedure. Aggregation was only checked by eye.</p> <p>6/ The suggestions provided by the Reviewers are very much appreciated.</p>
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	<p>Finding from DLS could be strengthen and validated by comparing data with use of complementary methods have been used for particle sizes of isolated lipoprotein fractions, such as high-performance liquid chromatography (HPLC), electron microscopy (EM) and nuclear magnetic resonance (NMR).</p>	<p>Indeed, we have ongoing experiments where we are separating purified Lp(a) subpopulations by SEC-MALS and FFF. Unfortunately, plasma samples from healthy donors have the great disadvantage of being limited in amounts, thus that these new experiments are being performed on different samples. In order to circumvent this issue, we are also planning to produce recombinant apo(a) and Lp(a) of given molecular weights in order to have access to indefinite homogenous samples. All these observations and future developments were introduced in the Discussion and Conclusions sections.</p>
<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments		