Reviewer's report

Title: Genetic variations and circulating levels of matrix metalloproteinase-9, plasma concentrations of tissue inhibitor of metalloproteinase-3 and B-type natriuretic peptide in patients with ventricular septal defect

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Reviewer: Trine Baur Opstad

Reviewer's report:

Review

In the manuscript “Genetic variation and circulating levels of matrix metalloproteinases-9, plasma concentrations of tissue inhibitor of metalloproteinase-3 and B-type natriuretic peptide in patients with ventricular septal defect” by Kun-Shan Cheng et al, the authors investigated the frequency of 3 MMP-9 polymorphisms in children with VSD and their effect on MMP-9 activity. Plasma levels of TIMP-3 and BNP were assessed accordingly.

The questions possessed by the authors are interesting. However some major concerns need to be raised.

Major Compulsory Revisions

The manuscript is overly long, and need to be compressed, especially the Results and Discussion sections. Some parts of the Discussion should preferentially be moved to the Introduction.

Do not repeat in the Results section what is already stated in the Method section.

Figure legends should not contain Results.

The results from the Zymography MMP-9 activity assay must be included in the Result section.

The Statistical analysis section should contain information about the correlation methods used in the presentation of data in Figure 1 and Figure 4. Did the author also perform Persons correlation in Figure 1 or is this a univariate regression model. Accordingly, is the line in Figure 1 a regression line or a simple line for trend? Why did the others not present an eventual regression line of trend line in Figure 4? Please specify and be more consequent in the presentation of data.

Did the authors check for Linkage Disequilibrium (LD) between the polymorphisms? Please include this data. As the MMP-9 -1562 C/T and R279Q are reported to be in LD this could have had an effect on the measured MMP-9 activity according to the R279Q polymorphism. Due to its location in exon 6, the polymorphism may alter MMP-9 activity, perhaps only detectable in those subjects exclusively bearing the minor allele of the R279Q polymorphism and not simultaneously being -1562 T-allele carriers. This should be discussed.

Why did the authors investigate TIMP-3 and not TIMP-1, or both, as TIMP-1 is
thought to bind with higher affinity to MMP-9 than TIMP-2 and TIMP-3 (Ref 5; Van den Steen et al), although TIMP-3 is reported to be involved in myocardial remodelling (Ref 29; Fedak PW et al).

Some references in the Reference list are not correctly referred to, i.e.: number 6 refers to intermedia thickness and not acute myocardial infarction (AMI), number 7 did not investigate patients with acute myocardial infarction, but patients with stable coronary artery disease. The same authors measured TIMP-1, and not Timp-3. Number 10 did not measure MMP-9 in relation to atherosclerosis, but MMP-1 and MMP-2 in relation to AMI. Number 21 did only refer to the MMP-9 -1562 C/T polymorphism and not several MMP-9 polymorphisms…. Number 22 did not measure TIMP-3……

All references must be thoroughly checked.

In the Result section where plasma MMP-9 and TIMP-3 levels are presented, please include BNP results also in this part as Figure 1, 2 and 3 are referred to in the text, and combine these two parts.

MinorEssential Revisions

The first sentence on page 7 “According to the manufacturer’s instructions, 100 ug of protein per sample was used” belongs perhaps to the Zymography method?

Be more precisely in the abstract, as BNP also was measured according to MMP-9 genotypes and VSD severity.

Table 1: MMP-9 and TIMP-3 measurements should also be included.

Table 2 should be corrected: For each polymorphism, 3 genotypes and minor allele frequency (MAF) should be presented. MAF frequency is the percentage of heterozygous divided by two, adding the percentage of the homozygous of the variant allele, then divided by 100 (i.e. for the MMP-9 -1562 C/T polymorphism, the T allele frequency in VSD subjects is (23.2 / 2 + 0) / 100 = 0.116.

The minor allele of the MMP-9 R279Q polymorphism is the G-allele, as the amino acid substitution Q (glutamine) is referred to in the literature to be the G-allele.

Is the Catalogue number for the TIMP-3 ELISA kit correctly referred? It was not found when searching on the R&D web sides

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests