Author’s response to reviews

Title: Can MiR-503 be used as a marker in diabetic patients with ischemic stroke?

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Klaus Brusgaard, PhD (Reviewer 1):

Some major points.

1. Performing power calculation you aim was 15 samples for each group. In the actual study the numbers are quite different especially the control group is rather small. Does this influence the results?

Groups II and III are both as control groups for group I. Expressional studies of Caporali et al. were performed on plasma and on muscular specimens taken from either amputated legs of diabetic patients (n=11) or calf biopsies of control group (n=11). We only extracted ‘d’ from this study and calculate our sample size based on this sample size formula.

\[(Z_{1-\alpha/2} + z_{1-\beta})^2 2(\text{SD})^2 \quad n = \frac{d^2}{\text{error}}\]
2. What is the distribution of men and woman in the different groups? You state that there is a considerable difference in the miR503 expression between sexes and it seems that the between sex difference is of the same magnitude as between the different groups. Did you perform correction for this?

Good point. We noted that mean age and sex distribution were not different among individuals in 4 groups (p>0.05) in the 2nd paragraph of the result. Sex distribution in each group is shown in also shown in table-1, which was not statistically different.

3. Reference 8 have been mixed up somehow.

Of course you are right. It is deleted.

Stefan Bilz (Reviewer 2): General comments:

The authors report on the expression of miR-503 in diabetic and non-diabetic patients with ischemic stroke, patients with diabetes and healthy controls. The main findings is that miR-503 expression in peripheral blood leucocytes is increased in patients with an acute ischemic stroke with diabetes when compared to controls without diabetes and in patients with diabetes and no acute disease when compared to healthy controls. In those suffering a stroke miR-503 expression decreases to levels of healthy controls in both groups. In both groups with no acute disease miR-503 expression remains stable but clearly increased in diabetic patients.

The rationale of the analysis is the previous finding that several miRNAs have been implicated in the pathophysiology of both diabetes complications and ischemic stroke. Specifically, miR-503 expression has been shown to be increased in the endothelial cells of diabetic mice and in the muscle tissue of amputated legs of diabetic patients with lower limb ischemia.

The results of this human in vivo study are interesting and extend those of previous research to suggest that the expression of miR-503 could serve as a diagnostic or prognostic biomarker in patients with diabetes and acute stroke.

Specific comments:

1. The small number of participants has to be discussed as a major limitation of the study.

Revised
2. Due to the small number of subjects in each group it is strongly advised that non-parametric statistical tests are employed unless a normal distribution of the data of interest has been proven.

Good comment, thank you. Revised.

3. Diabetes per se seems to be a major modifier of mir-503 expression. Fasting blood glucose is the only measure of glycemic control provided and positively correlated with mir-503 expression. The authors should indicate further measures of glycemic control, especially HbA1c.

The mean level of HbA1C was not different between groups (I) and (III). A column describing data about HbA1C is added in table-1.

4. Experimental work indicates that mir-503 expression is increased in endothelial cells under diabetic conditions. In this study, mir-503 expression is increased in peripheral blood leucocytes. Please comment.

Although the concentration of miR in endothelial cells is higher, miR leaks into plasma and we can rely on plasma level as an indicator of the miR amount inside the cells. Many studies measured the level in blood. Our supported experiment (Caporali’s study) got the sample from blood in last part of the study, which was performed on human beings.

5. Due to the obviously prominent role of endothelial cells in the regulation of mir-503 expression it should be indicated wether there is an association between microvascular complications (e.g. microalbuminuria, retinopathy) and mir-503 expression.

Good point of view. Our study was a pilot study with small sample size. Our second goal is to assess microvascular complications in larger amount of people but as this study was not funded we can not extend our primary goals.

6. The authors report a positive correlation of plasma cholesterol concentrations with mir-503 expression. Please provide a rationale/discuss this observation.

Hyperlipidemia is a risk factor for both diabetes and stroke. There is no evidence that increased level of cholesterol affect miR-503 expression independently, but many other miRs are studied to show their role in pathogenesis of hyperlipidemia. We did not find any relationship between miR level and cholesterol level inside groups, as the number of patients in groups was small. So we cannot make a conclusion about cholesterol level due to lack of data for analysis.
7. It is concluded that antagonizing mir-503 may be a therapeutic option in patients with ischemic stroke. From the data presented, this statement seems very premature and should not be part of the conclusions.

Revised. It is not a conclusion from our study. It is just a hypothesis according to literature review, not limited to miR-503.

8. In patients with diabetes mir-503 Expression decreases to control levels 3 months after stroke. Since glycemic control is a major regulator, HbA1c and fasting blood glucose 3 months after the stroke should be presented.

Revised. Unfortunately we do not observe HbA1C after 3 months, but the mean level of FBS was not different after 3 months.

9. The discussion is quite lengthy and not easy to read. The manuscript should be edited by a native English speaking person.

Revised