Putative miRNA biomarkers of insulin resistance and the effect of metformin: data from the CAMERA trial

T. ALRamah1, K. Pinel1, D. Preiss2, N. Sattar1, J. Logue1, P. Welsh1; 1University of Glasgow, Glasgow, 2University of Oxford, Oxford, England, UK.

Background and aims: Several circulating miRNAs have been reported to be associated with insulin resistance (IR) and are considered as potential diagnostic biomarkers, although studies have generally been limited by small size. It is also not clear whether these associations are causal. We aimed to 1) validate cross sectional associations of targeted miRNAs within a large study and 2) to explore the effect of metformin on these miRNAs.

Materials and methods: The CAMERA study was a randomised, placebo-controlled double-blinded trial performed in Glasgow, UK (2009-2012). 173 patients aged 35-75 on statins with coronary heart disease and large waist circumferences (but without diabetes) were randomly assigned to metformin or placebo (1:1) and followed for 18 months. miRNAs were extracted from 308 paired stored samples from 154 participants (taken at baseline and after 18 months of treatment). The expressions of mir-221, 222, 144, 155, 192 and 193b (targeted based on previous literature) were measured using real time quantitative polymerase chain reaction (RT-qPCR). Samples from each patient were run simultaneously. Spike-in of cel-miR-39 was used as a quality control. We used linear regression of CT values for cross sectional analyses, and the 2-ΔΔCT method to investigate the randomised effect of metformin.

Results: In cross sectional analysis at baseline, mir-144 showed the strongest association with markers of insulin sensitivity: in models adjusting for age and sex, for every unit increase in HOMA-IR, mir-144 was 10.2% (95% CI 2.5% - 17.2%, $p<0.05$) lower and for a 1 mmol/L increase in fasting plasma glucose (FPG), mir-144 was 34.9% (95% CI 15.1% - 50%, $p<0.01$) lower. Other miRNAs showed variable associations with markers of insulin resistance and glycaemia, but no miRNA was associated with BMI. Mir-192 and 193b showed strong associations with liver enzymes. For every 10-unit increase in ALT, mir-192 was 24.7% (95% CI 16.5% - 32.2%, $p<0.001$ lower while mir-193b was 34.9% (95% CI 28.8% - 40.1%, $p<0.001$) lower. For every 10-unit increase in GGT, mir-192 (95% CI 2.7% - 8.6%, $p<0.001$) was 6% lower and mir-193b was 8% (95% CI 5.4% - 10.5%, $p<0.001$) lower. Adjustment for body fat did not attenuate these associations. Over 18 months, metformin reduced weight by 3.3 kg, FPG by 0.3 mmol/L and HOMA-IR by 0.81 units versus placebo. However, metformin showed no effect on the expression of mir-221, 222, 144, 155, 192 and 193b ($p=0.91$, 0.51, 0.25, 0.97, 0.89 and 0.69 respectively). From observational data, this effect would be expected to translate into an ~8% increase in miRNA-144, which may not be of clinical relevance.

Conclusion: In this, one of the first studies to investigate the effect of a randomised intervention on miRNA expression, we observed broadly expected cross-sectional associations of targeted miRNAs with bio- markers of metabolic risk in a non-diabetic population with coronary heart disease but without diabetes. These data support the putative utilities of miRNAs as reproducible biomarkers, but more RCTs should explore the effect of interventions on their expression.

Clinical Trial Registration Number: NCT00723307 Disclosure: T. ALRamah: None.