MIR-146a AND -155 ARE INVOLVED IN FOXO-1 REGULATION AND NON ALCOHOLIC FATTY LIVER DISEASE (NAFLD) IN CHILDHOOD OBESITY

L. Montanini[1], P. Lazzeroni[1], C. Sartori[1], V. Nobili[2], P. Crafa[3], S. Bernasconi[1], M.E. Street[1,4]

P1-D1-112

1. Dept. of Paediatrics and Maternal Care, University Hospital of Parma, Parma, Italy
2. Hepatology, Gatroenterology and Nutrition Dept., "Bambin Gesù" Hospital and Research Institute, Roma, Italy
3. Dept. of Pathology, University Hospital of Parma, Parma, Italy
4. Dept. of Paediatrics and Research and Statistics, S. Maria Nuova Hospital and Research Institute, Reggio-Emilia, Italy

INTRODUCTION

NAFLD is the most common chronic liver disease in childhood. This condition is associated with insulin resistance.
FOXO-1 is a key regulator in insulin signalling and in intracellular adipogenesis, and it has been shown to be implicated in NAFLD; its expression has been correlated with liver function in obese patients.
MicroRNAs (miRNA) are small non coding RNA molecule involved in post-transcriptional regulation of gene expression.
We have previously identified a group of miRNAs involved in epigenetic regulation of FOXO-1 gene. In particular miR-146a and miR-155 seem to be related with the control of glucose homeostasis.

AIMS

In the first phase of our study we assessed miR-146a and miR-155 expression in liver tissue from NAFLD subjects.
We then aimed at assessing these miRNAs in serum and investigated relationships with BMI and adipose tissue distribution besides relationships with the presence or absence of NAFLD in order to verify whether they could become eventually predictors and/or markers of this complication.

MATERIALS AND METHODS

Tissue study

MiR-146a and miR-155 were extracted from paraffin embedded liver biopsies taken from 10 subjects with NAFLD and comparable controls; they were subsequently quantified by TaqMan microRNA Assays and normalized with respect to RNU48 (Applied Biosystems, FosterCity, USA).

Serum studies

We enrolled 25 obese children, with and without NAFLD, attending our Paediatric Endocrine Clinic in Parma University Hospital (Table 1). NAFLD was diagnosed by liver ultrasound undertaken by a single operator.
Total RNA from serum was extracted using MirVana PARIS kit (Ambion, Austin, USA), following the manufacturer's protocol. MiR-146a and miR-155 were quantified by TaqMan microRNA Assays and normalized with respect to miR-16 and miR-93, as housekeeping miRNAs. dCts were normalized with respect to an appropriate pool of dCt controls of comparable age, sex and pubertal stage. Relative gene expression was then presented as fold change (Log_{10}) (Table 2).

Statistical analysis

Standard statistical analysis was performed using statistical package SPSS 18.0 as appropriate.

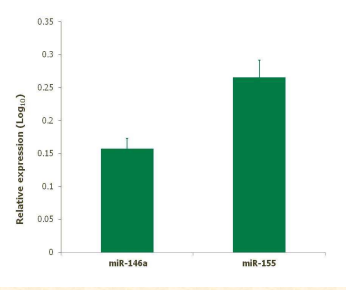
Table 1.

	Steatosis	Non steatosis
Number of subjects	16	9
Age	11,89 ±0,86 yr	11,81 ±1,0 yr
Sex	8M, 8F	5M, 4F
Pubertal stage	5 prepubertal, 6 in puberty, 5 postpubertal	2 prepubertal, 3 in puberty, 4 postpubertal
BMI SDS (Cole)	3,41	3,15
WtC/ht ratio	0,68 ±0,06	0,63 ±0,05

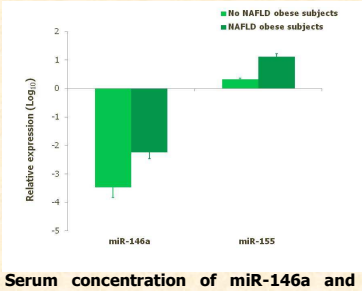
Table 2.

Number of subjects	22
Age	12,1 ±1,3 yr
Sex	12F, 11M
BMI SDS (Cole)	0,56 ±0,66
Pubertal stage	10 prepubertal, 8 in puberty, 4 postpubertal

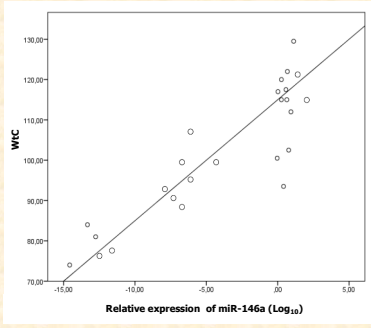
RESULTS



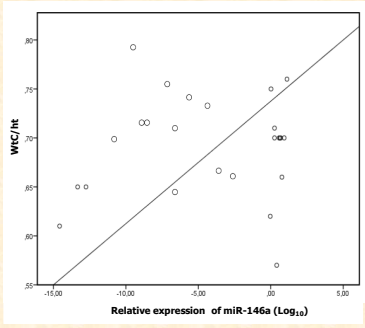
Expression levels of miR-146a and miR-155 in bioptic tissue samples.
Both miRNAs were over-expressed in subjects with NAFLD with respect to controls.



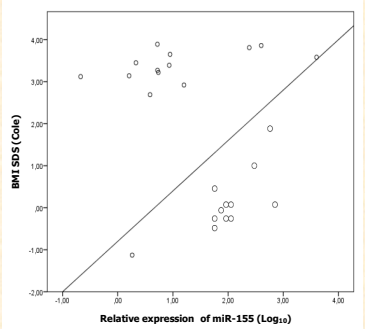
Serum concentration of miR-146a and miR-155 in obese subjects.
Relative expression of both microRNAs was opposite: miR-146a was down-regulated, whereas miR-155 was over-expressed in obese subjects with and without NAFLD.



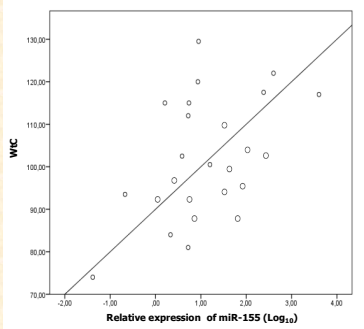
Correlation between miRNA146a and waist circumference (WtC).
R: 0.65; P=0.01



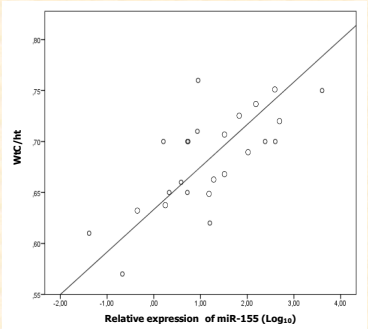
Correlation between miRNA146a and waist circumference/height ratio (WtC/ht).
R: 0.52; P=0.05



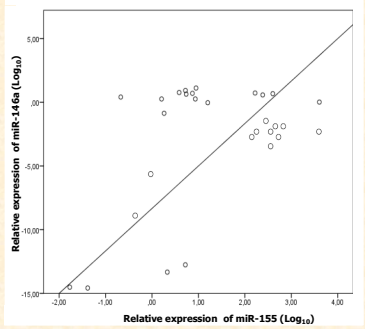
Correlation between miRNA155 and BMI SDS (Cole).
R: 0.55; P=0.04



Correlation between miRNA155 and waist circumference (WtC).
R: 0.71; P=0.04



Correlation between miRNA155 and waist circumference/height ratio (WtC/ht).
R: 0.64; P=0.01



Correlation between miRNA155 and miRNA 146a.
R: 0.47; P=0.04

CONCLUSIONS

MiR-146a and miR-155 were significantly different in serum of obese children with respect to controls. In obese children having NAFLD, the relative gene expression of both miRNAs was significantly greater. Further investigations are necessary to verify whether these miRNAs could become markers/predictors of NAFLD in obese children.