Cord blood microRNA alterations at birth predate persistent childhood

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Introduction

- Childhood obesity and rapid weight gain in infancy can be detrimental to the wellbeing of the child and increases the risk of metabolic disorders, cardiovascular disease, and type 2 diabetes (T2D) later in life.
- To our knowledge, there are currently no definitive predictors at birth for infants predisposed to rapid weight gain or development of obesity.

Aim To examine alterations in cord blood miRNA of infants preceding the development of rapid weight gain and childhood obesity.

Method

Cord blood was collected at delivery in infants from the Cork Baseline Birth Cohort Study. Weight for length (WFL) was measured serially from birth to 5 years. Cord plasma miRNA was profiled using next-generation sequencing (NGS) and *in silico* analysis. Cases were defined as infants with rapid early weight gain (change in WFL z-score >1) and BMI in the obese range (>98% BMI) at 2 years. Control infants had a change in WFL z-score between -0.5 to 0.5 and a normal range BMI at 2 years. Both cases and controls were assessed for persistent obesity at 5 years. Statistics were performed using SPSS 24.0 and R v3.2.4. Functional targets, gene ontology and predicted pathways were generated using DNA Intelligent Analysis (DIANA, <http://snf-515788.vm.okeanos.grnet.gr>), miRPath v3.0 and Cytoscape v3.2.

Results

A nested cohort of 20 cases and 16 controls were identified. NGS identified 73 altered miRNAs at 2-years and 38 altered miRNAs at 5-years. Following initial alterations across groups, VIP rankings were given to the top 15 miRNAs, at two years, fig. 2B, and five years, fig. 2C. From these, 12 miRNAs were overlapping at both two years and five years. The 12 miRNAs altered across both the two-year group and the five-year group were investigated for functional validated targets, fig. 3A, gene ontology and pathway analysis, fig. 3B-C.

Table 1. Clinical Demographics.			
	Control <i>n</i> = 20	Case <i>n</i> = 16	P-Value
Maternal Characteristics			
Age at delivery (years)	30.5 (27.5 – 32.0)	31.0 (29.0 – 34.0)	0.765
BMI at 14-16 wks gestation (kg/m ²)	23.90 (21.98 – 26.13)	25.35 (23.98 – 28.98)	0.077
Third-level education (Y/N)	18/2	15/1	0.863
Smoked in pregnancy (Y/N)	1/19	3/13	0.498
Infant Characteristics			
Sex (M/F)	11/9	7/9	0.582
Gestational age (wk)	40.6 (39.7 - 41.3)	40.6 (40.1 - 41.8)	0.987
Birthweight (g)	3785 (3183 - 4100)	3725 (3405 - 3968)	1.000
Change in WFL SDS			
0 - 12 months *	-0.05 (-0.07 - -0.01)	2.00 (1.84 – 2.83)	<0.001
Child Characteristics at two years			
Assessment age (years)	2.10 (2.08 – 2.16)	2.11 (2.08 – 2.15)	0.962
BMI SDS at two years *	0.82 (-0.28 – 1.13)	2.36 (2.22 – 2.75)	<0.001
Child Characteristics at five years †			
	<i>n</i> = 16	<i>n</i> = 11	
Assessment age (years)	5.06 (4.99 – 5.15)	5.07 (4.97 – 5.19)	0.904
BMI SDS at five years *	0.14 (-0.41 – 0.42)	1.91 (1.40 – 2.50)	<0.001

Clinical demographics in the Baseline rapid growth cohort. Median (IQR). M = Male, F = Female, wk = week, g = grams, SDS = standard deviation score, WFL = weight for length. * P-Value < 0.05. † *n* = 4 controls, *n* = 5 cases lost to follow-up.

Conclusions

- This pilot study has shown that umbilical cord plasma-based miRNA levels are altered preceding infants with rapid weight gain that develop childhood obesity.
- The 12 highest-ranking miRNAs predictive of 2-year and 5-year outcome have been previously reported in studies of diabetes mellitus, myocardial dysfunction, obesity, adipogenesis, and cholesterol metabolism.
- Future studies, should further validate these miRNAs with qPCR; validated miRNAs should also investigate temporal changes over the course of early life.

Figure 1. Flowchart of next-generation sequencing (NGS) results. Criteria for NGS analysis and differential miRNA expression using two year and five-year outcome, and alterations seen across both groups. RWG = Early rapid growth.

Figure 2. Discriminant analysis with partial least squared-discriminant analysis (PLS-DA) of total microRNA discovery and random forests variable important plot (VIP) of top 15 microRNA at two years and five years. A. PLS-DA analysis accounts for 77.3% of cases (*n* = 20) and controls (*n* = 16) at two years. B. VIP ranking of top 15 microRNA up to two years. 14 increased miRNAs (red box) and 1 decreased (green box) in cases. C. PLS-DA analysis accounts for 65.2% of cases (*n* = 16) and controls (*n* = 11) at five years. D. VIP ranking of top 15 microRNA up to five years. 13 increased miRNAs (red box) and 2 decreased miRNAs (green box) in cases. * Overlapping microRNA at both two years and five years. Left (green) ellipse in A and C indicates 95% confidence interval (CI) of controls; right (red) ellipse in A and C indicates 95% CI of cases.

Figure 3. Functional miRNA targets from 12 overlapping cord blood miRNAs. A. Validated gene miRNA regulatory networks. The white nodes are miRNAs, and the blue nodes were validated miRNA targets. The edge connecting two nodes are indicative of validated interactions using miRWALK 2.0. B. Top 10 significantly associated gene ontologies from validated miRNA targets, using miRPath 3.0. C. Validated miRNA-mRNA target associated with KEGG pathways cluster dendrogram, using miRPath 3.0. P-value < 0.05 was considered statistically significant, and FDR correction was applied.

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(INFANT) and was funded in part (or whole) by Science Foundation Ireland (12/RC/2272)