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Supplementary Methods

Collection and processing of placental sample sets: Placental sampling has recently been described in detail (Kasak et al., 2015; Sõber et al., 2015; Reiman et al., 2017; Kikas et al., 2019). The placentas collected at term or during 2nd trimester medically induced abortion due to maternal medical risks were stored at +4°C after vaginal delivery or C-section, and processed further within one hour. A full-thickness block of 2x2 cm was taken from the middle region of each placenta quarter. The excision site was chosen away from the umbilical cord insertion site, large vessels, and any visible or palpable infarction, hematoma or damage. Samples were washed with 1x Phosphate Buffered Saline (PBS) to remove maternal blood and divided for both DNA and RNA extraction at a later date. The block was further longitudinally dissected through all layers. Sections of 1 g or 100 mg of tissue for RNA extraction were placed into 10 ml or 1 ml RNAlater solution (AM7021; Thermo Fisher Scientific). Samples were kept at +4°C for 1-3 days in RNAlater and then stored at -80°C until RNA extraction. Placental samples used for DNA extraction were placed in dry cryovial immediately after excision and washed with 1x PBS. Placental DNA was extracted using NucleoSpin Tissue kit (Macherey-Nagel, Germany) according to the manufacturer's instruction. All samples were collected by the same medical personnel.

Samples of the 1st trimester placentas were obtained immediately after elective (surgical) termination of pregnancy. The procedure to purify placental chorionic villi is described previously by Sõber et al., 2016 and Kasak et al., 2017. Samples were washed with a solution containing 15 ml Dulbecco's PBS, 0.3 ml penicillin-streptomycin solution 10000U/10000µg/ml and 2 drops of heparin (5000U/ml). Maternal cells were removed under a stereomicroscope (Discovery V8, Zeiss) and chorionic villi containing both cytotrophoblast and syncytiotrophoblast cells of fetal origin were placed into a dry tube (for DNA analysis) or RNAlater solution (for RNA analysis) and stored at -80°C. For the 1st trimester samples, karyotyping was applied to confirm normal male or female karyotype.

RNA and DNA extraction protocols: All placental samples had been kept at -80°C until DNA/RNA isolation. For total RNA isolation, a tissue sample was thawed on ice and a 200–300 mg slice (mostly comprised of chorionic villi) was cut from the middle of the sample. Total RNA was extracted from 200-300 mg of homogenized placental tissue using TRIzol reagent (Invitrogen, Life Technologies). Samples were purified with RNeasy MinElute Columns (Qiagen, Netherlands) when used for Total RNA sequencing. Purity level and concentration of isolated total RNA was measured using NanoDrop® ND-1000 UV-Vis spectrophotometer (Thermo Fisher Scientific Inc., USA) and RIN (RNA integrity number) was estimated using Agilent 2100 Bioanalyzer (Agilent Technologies, USA). DNA was extracted using NucleoSpin Tissue kit (Macherey-Nagel, Germany) according to the manufacturer's instructions.

Total RNA sequencing (RNA-Seq) and data processing: Placental RNA-Seq of the samples analyzed in the current study has been described in detail elsewhere (Sõber et al., 2015; Reiman et al., 2017). The RNA-Seq library preparation, RNA sequencing and raw data processing were conducted at the FIMM Sequencing Laboratory, University of Helsinki, Finland. High-quality DNA-free total RNA (5 µg) was used for rRNA depletion (Ribo-Zero™ rRNA Removal Kit, Epicentre) followed by cDNA synthesis (Life Technologies). Library preparation was performed using the Nextera® Technology (Illumina). Total RNA sequencing was performed on Illumina HiSeq2000 with 46 bp paired-end reads (101 bp for two samples).

The total RNA-Seq datasets were processed using an up-to-date bioinformatics pipeline (Kumar et al., 2017) at the FIMM NGS bioinformatics unit, University of Helsinki. Briefly, the raw reads were corrected for low quality, Illumina adapters, and short length using Trimmomatic (Bolger et al., 2014) with the following parameters: leading: 3, trailing: 3, sliding window: 4:15, illuminaclip 2:30:10, and minlen: 36. Quality trimmed paired-end reads were then aligned by STAR (Dobin et al., 2013) to the human reference genome (GRCh38) with the guidance of EnsEMBL v82 reference gene models. Default 2-pass per-sample parameters were used, except that the overhang on each side of the splice junctions was set to 99. Picard tools (http://picard.sourceforge.net) were used to sort and mark duplicate reads. Reads were assigned to genomic features using SubRead (Liao et al., 2013). Default parameters were used, except that in the feature summations reads were allowed to be assigned to overlapping genome features. Quality control analysis was performed using the RNA-SeQC (Deluca et al., 2012) and FastQC (Andrews, 2010) with default settings and supported by the GRCh38 associated references files.

Genome-wide genotyping of placental samples: Genome-wide genotyping of placental samples investigated for the miRNome profile in this study was performed using Illumina HumanOmniExpress-12-v1 BeadChip (>733,000 SNPs, median spacing 2.1 kb)(Kasak et al., 2015; Kikas et al., 2019; Pilvar et al., 2019). Samples were genotyped with an average overall call rate >99% per individual per genotype. Normalized signal intensity data were obtained through Illumina GenomeStudio software. SNPs were excluded if they deviated from Hardy-Weinberg Equilibrium (HWE; $P<1\times10^{-6}$) or had no minor allele carriers in our dataset. After data filtering, 661,354 genotyped SNPs were taken forward to the next step.

REPROMETA and HAPPY PREGNANCY cohort samples for genetic association testing between miR-eQTLs and pregnancy traits: The study utilized samples from Estonian REPROMETA (full study name "REPROgrammed fetal and/or maternal METAbolism"; recruitment 2006–2011) and HAPPY PREGNANCY (full: "Development of novel non-invasive biomarkers for fertility and healthy pregnancy"; 2013-2015) data sets. Both studies were approved by the Ethics Review Committee of Human Research of the University of Tartu, Estonia (Permissions No 146/18, 27.02.2006; 150/33, 18.06.2006; 158/80, 26.03.2007; 221/T-6, 17.12.2012; 286/M-18, 15.10.2018). All study participants were recruited, and the study material was collected at the Women's Clinic of Tartu University Hospital, Estonia. Written informed consent to participate in the study was obtained from each individual prior to recruitment. The study was carried out in compliance with the Helsinki Declaration and all methods were carried out in accordance with approved guidelines. All participants were of white European ancestry and living in Estonia.

The REPROMETA study recruited family duos or trios (mother, father and placenta) (n=366 families) before or shortly after delivery of a singleton newborn (**Table S3**). The study was designed to include well-defined, clinically diagnosed diverse scenarios of pregnancy outcomes at term (36-42 gestational weeks). The recruited cases represented five clinical subgroups: delivery of a small-for-gestational-age (SGA, birth weight <10th centile) or large-for-gestational-age newborn (LGA, >90th centile), cases of maternal gestational diabetes (GD) or late-onset preeclampsia (PE), and normal term pregnancies (birth weight 10th-90th centile). Maternal-newborn clinical and epidemiologic data were documented retrospectively from self-reported questionnaires and medical records, the collected biological material included placental tissue and parental blood samples. Cases with documented fetal anomalies, chromosomal abnormalities, families with a history of inherited diseases, and patients with known pre-existing diabetes mellitus, chronic hypertension, and chronic renal disease were excluded.

The HAPPY PREGNANCY study prospectively recruited a cohort of 2,334 pregnant women during their first antenatal visit without any pre-selection criteria (**Table S3**). Longitudinal clinical data for the course and pregnancy outcome were extracted from medical records; epidemiological data including reproductive history, parental lifestyle were collected from three questionnaires across gestation.

In both studies, SGA and LGA newborns were defined based on the Estonian Medical Birth Registry (Sildver et al., 2015). The diagnosis of PE and GD followed the international guidelines at the time of recruitment (Metzger, 2010; American College of Obstetricians and Task Force on Hypertension in Pregnancy, 2013). All PE cases represented the severe form of preeclampsia, diagnosed as extreme hypertension (systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥110 mmHg), combined with proteinuria of ≥5 g in 24 hours or neurological symptoms. GD was diagnosed when a 75 g oral glucose tolerance test (OGTT) performed at 24–28 weeks of gestation revealed either a fasting venous plasma glucose level of ≥5.1 mmol/l and/or at 1h and 2h later plasma glucose level of ≥10.0 mmol/l and ≥8.5 mmol/l glucose, respectively.

TaqMan qPCR: TaqMan genotyping was performed for the REPROMETA (n=326) and HAPPY PREGNANCY (n=1,772) study cases with available placental tissue. All genotyping reactions were performed in 384 micro-well plates in ViiA 7 Real-Time PCR System (Applied Biosystems, Life Technologies). TaqMan probes were used for rs12985296, rs7046565 and rs12420868, experimental conditions as recommended by the protocol (Applied Biosystems, Life Technologies; **Table S4**). Genotype was called using ViiA 7 QuantStudio Real-Time PCR software (Applied Biosystems, Life Technologies).

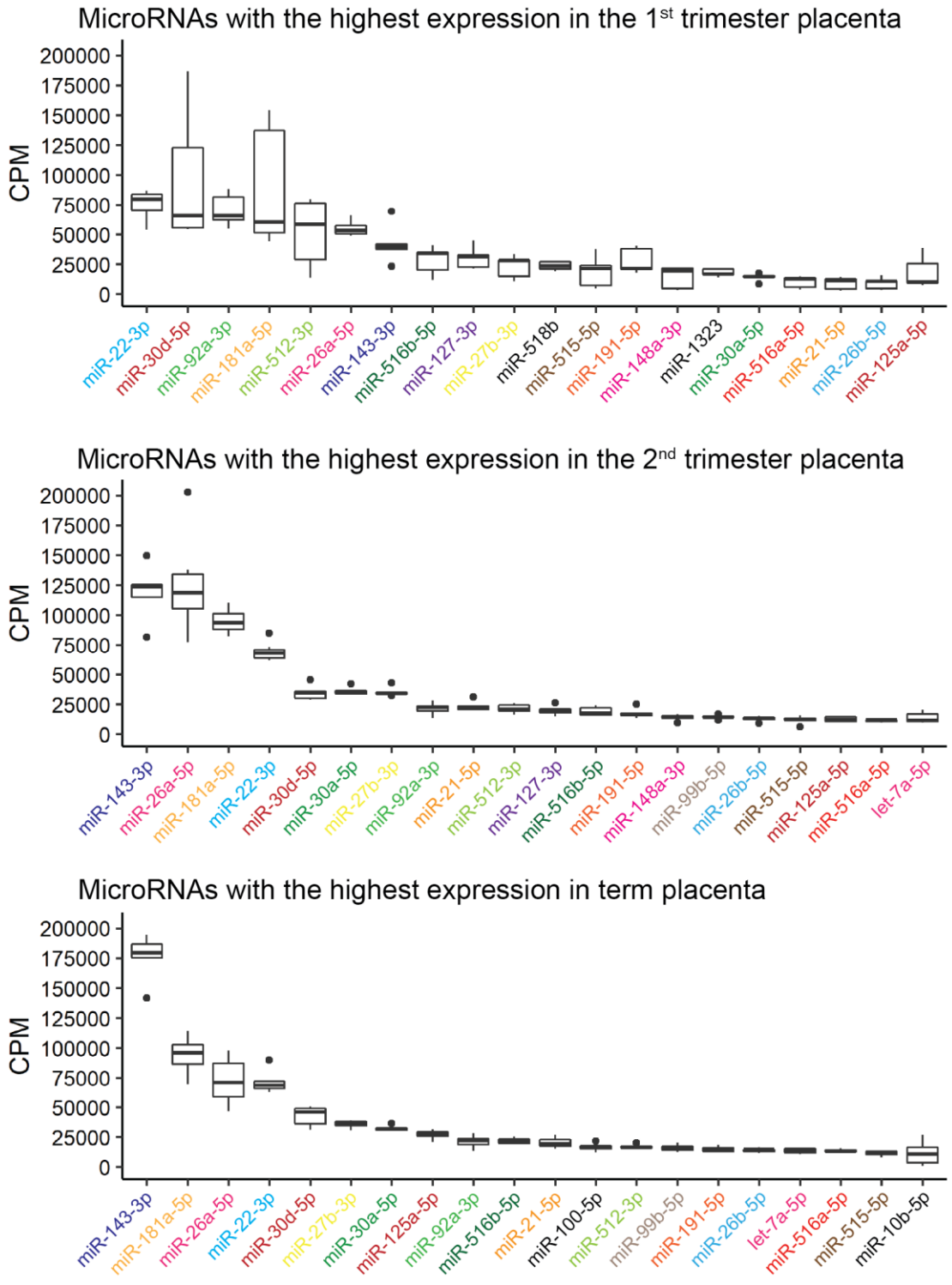

Figure S1. Top 20 placental microRNAs with the highest expression level in the 1st and 2nd trimester of pregnancy, or at term. miRNAs are color-coded to facilitate easier identification of the corresponding data in each subfigure. CPM, counts per million reads mapped; miR, microRNA.

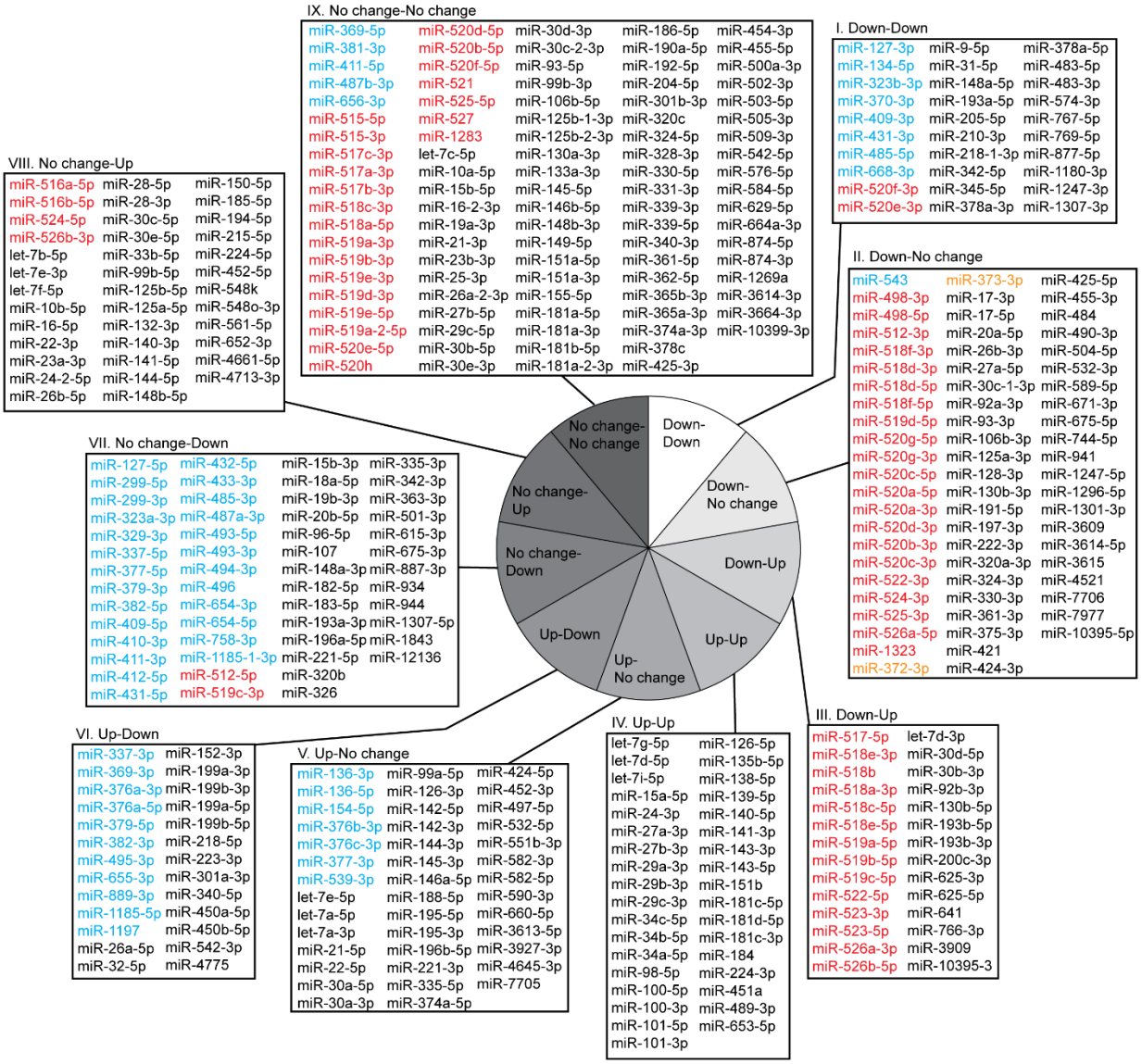

Figure S2. Distribution of 417 analyzed placental microRNAs according to the expressional dynamics patterns I-IX across pregnancy. Each microRNA was assigned to one of the nine patterns of expressional change based on the differential expression testing results between the miRNomes of 1st and 2nd trimester (Up, Down or No change) and 2nd trimester and term placenta (Up, Down or No change) (**Table 2**). For example, 'No change-Up' refers to miRNAs that do not show differential expression between 1st and 2nd trimester, but are significantly up-regulated in term placentas. Normalized expression values of each individual miRNA in the 1st trimester, 2nd trimester and term placentas are provided in **Table S1** and differential expression testing results between trimesters are shown in **Tables S7-S8.**

Red, blue and yellow fonts refer to miRNAs belonging to the placenta-specific miRNA clusters C19MC, C14MC and miR-371–373, respectively.

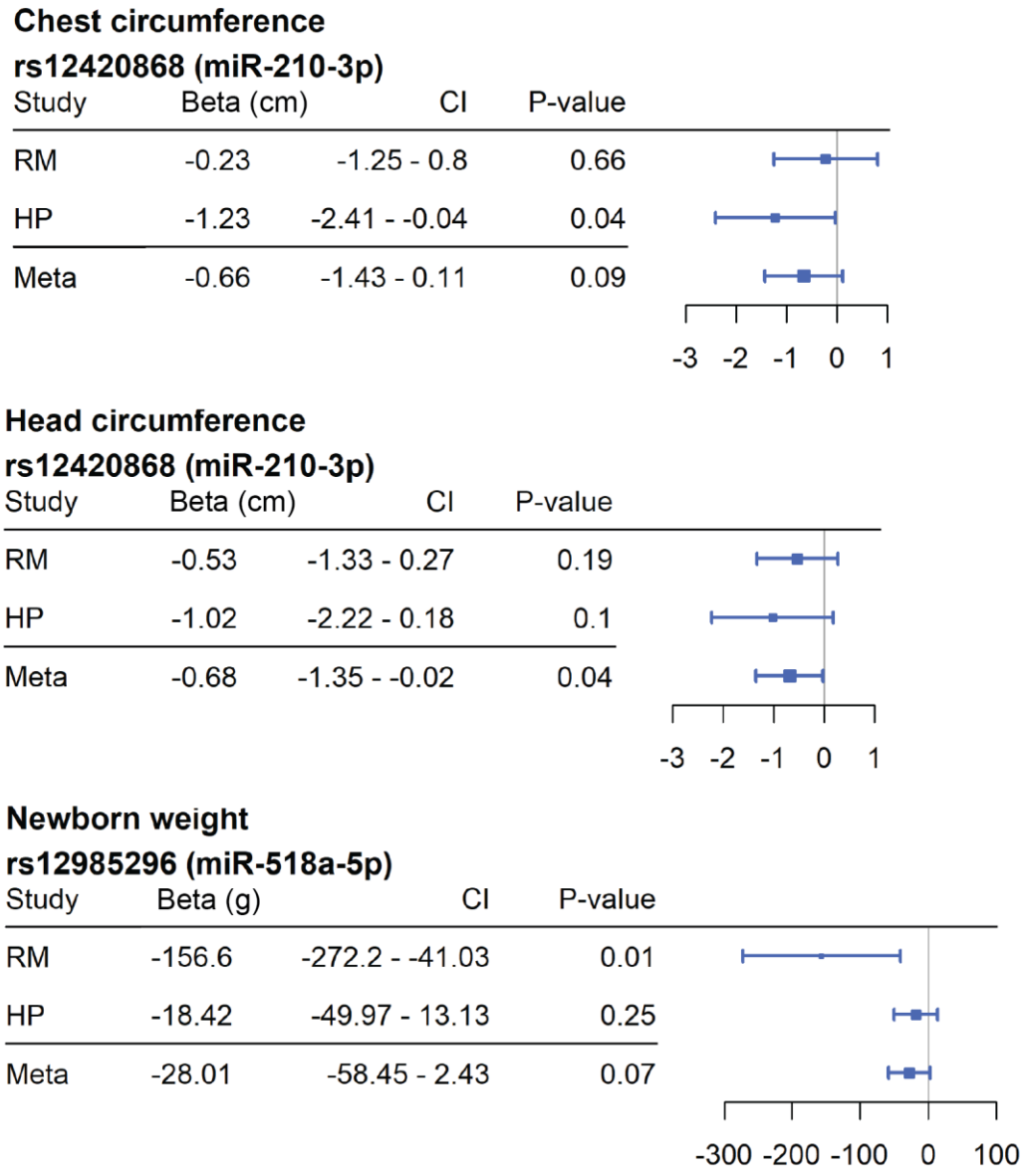

Figure S3. Association testing between placental miR-eQTLs and newborn traits: results with nominal *p*-values <0.05 in REPROMETA, HAPPY PREGNANCY or their combined meta-analysis. microRNA expression Quantitative Trait Locus (miR-eQTL) represents an SNV that modulates the expression level of the flanking miRNA (indicated in brackets). Association testing was carried out between placental genotypes of the REPROMETA (RM, n=326) and HAPPY PREGNANCY (HP, n=1,772) study samples. All tests were performed in PLINK ver. 1.07 (Purcell et al., 2007). Meta-analysis of RM and HP datasets (n=2,097) was implemented in R package meta (ver. 4.15-1)(Balduzzi et al., 2019), under fixed-effect model. CI, confidence interval; Beta, effect of the SNV expressed in the unit of the measurement.

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