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Differential gene expression (DGE) analysis was applied to detect relationships between distinct maternal diagnoses and UCB gene expression levels using the limma package (Ritchie et al., 2015). The covariates maternal tobacco use, maternal alcohol use, RIN, batch, biological sex, gestational age, mode of delivery and ethnicity were included in all models to adjust for their potential confounding influence on UCB gene expression between main group effects, and the significance threshold was a nominal P-value < 0.05. This P-value threshold was used to yield a sufficient number of genes to include within functional annotation and gene network analyses, described below. Power and sample size was estimated using the R package ssize.fdr (Orr and Liu, 2015).

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# 6223127

There are dozens of power and sample size calculations available in R (Supplementary File 9), but they are scattered among dozens of different package libraries: samplesize, ssize.fdr, pwr, powerpkg, powerAnalysis, powerbydesign, Sample.Size, samplesize4surveys, etc. There are also basic power and sample size functions in the default stats library. Often specific power and sample size libraries will address specific types of statistical tests and experimental designs, while other libraries may be more general. Several examples are shown using the functions power.t.test() and power.anova.test() in Supplementary file 9.

In the first examples, the power.t.test() is used to calculate 3 single estimates for sample size, power and delta. Then power.t.test() is used to compute 9 estimates of sample size (Fig. 6d), power (Fig. 6e) and delta (Fig. 6f) to plot some basic power and sample size curves. Likewise, in the second series of examples, the power.anova.test() function is used to calculate 3 single estimates of sample size (Fig. 6g), power (Fig. 6h) and delta (Fig. 6i), before multiple sequences of estimates are calculated and plotted. Finally, ssize.twoSampVary() from the ssize.fdr package library is used to calculate power estimates for a typical gene expression microarray analysis (Fig. 6j).

# 5737945

The power calculation was performed with the ssize.fdr R package [8]. Based on the above described experiment of miRNA expression levels between CAD patients and controls, a common standard deviation of 0.56, and an estimated proportion of non-differentially expressed miRNAs of 0.83 with a false discovery rate (FDR) controlled at 10% was used. The true difference between mean expressions in the two groups as well as the standard deviations of expressions were assumed to be identical for all miRNAs. The common value for the standard deviations was estimated from the data and set conservatively to the 90th percentile of the gene residual standard deviations. The proportion of non-differentially expressed genes π0 was estimated using the qvalue function (qvalue R package) on the vector of p-values obtained from the differential expression analysis.

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For the discovery cohort used to study gene expression, a mixed effect linear regression model was fitted for each probe and genotype using R (RStudio version 0.99.489) lme4 package [38], after correcting for gender, age, diagnostic status and family or twin status effects as a covariate. This analysis was performed separately but identically for the discovery cohort and the replication twin cohort. We used the false discovery rate (FDR) method in place of a family-wise error rate (FWER). FDR is widely applied for microarray analyses because it allows more genes to be extracted for further exploration, and was performed using the qvalue package in R [39] to estimate the FDRs of q ≤ 0.05. The post hoc power of our small familial discovery cohort to detect gene expression changes was estimated using R package ssize.fdr [40]. The GTEx database was mined using its own in-built test procedure, entering in a list of gene IDs to be tested against our SNPs of interest. Data from whole blood was used in order to replicate only those genes identified as significantly altered in their gene expression levels at a cut-off of p ≤ 0.05. When testing for replication, probes significant (p ≤ 0.05) in the discovery cohort prior to application of FDR were studied. See figure 1 for a flow chart of analysis.

# 5569060

The Northern Sweden Health and Disease Study (NSHDS) comprises of 94,630 sampling occasions from 74,690 unique individuals. Within the EnviroGenomarkers project archived blood samples and exposure/health data were derived from -at that time- healthy subjects, including 229 future cases of B-cell lymphoma and 327 controls of the prospective NSHDS. No subject was diagnosed with lymphoma within less than two years of blood sample collection. Cases were matched to healthy controls by gender, age (+/− 2.5years), hospital and date of blood collection (+/− 6 months)11. For this study we randomly selected 226 buffy coat samples for integrated miRNA and transcriptome analysis (Table 4, Supplementary Figure S1). To determine the statistical power of the sample size we conducted a power calculation for microarray experiments using the R package “ssize.fdr”41. Accordingly, a minimum of 190 subjects were required to achieve 80% power. For the 207 subjects that were analyzed in our study we calculated 84% power at the 5% significance level (FDR corrected).The EnviroGenomarkers project and its associated studies and protocols were approved by the Regional Ethical Review Board of the Umea Division of Medical Research and all participants gave written informed consent. This study was conducted in accordance with the approved guidelines and regulations.Table 4

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Power calculations based on primary data in [1]. Unadjusted power and total sample size calculations were done in R v3.2.2 (via the pwr.t.test function in the pwr package [7]. Effect sizes were Cohen’s d. The difference in means was the corresponding log2 fold change. SD was estimated as the pooled standard deviation for a given comparison. These power calculations did not assume false discovery rate (FDR) adjustments because we were concerned that it would be overly conservative and remove potential true positives (tests were not necessarily independent). Further, the inclusion of the false positives that the FDR corrections would have removed should actually increase our odds of identifying any transgenerational effects (whether they were true or false positives), but even under these more relaxed conditions, none could be established. FDR corrected calculations are also displayed in the last two columns. These were done in R via the ssize.twoSamp function from the ssize.fdr package [8]. A true positive ratio of 0.05 was assumed for the FDR calculationsComparison numberSample size (n treated + n control)Pooled AVG STDVPower for log(2) changePower to detect 50 % changeEffect size for 50 % difference power calculationNumber of samples to detect 1.5-fold change with power = 0.9Number of samples to detect 1.5-fold change with power = 0.8Number of samples to detect 1.2-fold change with power = 0.9Number of samples to detect 1.2-fold change with power = 0.8Number of samples to have 5 % FDR in detecting 1.2-fold changeNumber of samples to have 5 % FDR in detecting 1.05-fold change160.161.000.923.7566181432370260.161.000.913.6466181434392360.151.000.943.9666161430332460.161.000.903.5786201634408560.151.000.933.8066181432362660.161.000.903.56862016344101340.150.890.553.93661614303381440.150.880.533.78661814323641760.151.000.933.83661814303541860.161.000.923.71661814323781960.141.000.974.26661412262882060.151.000.943.90661614303422160.151.000.954.02661612283222260.151.000.933.84661814303522940.150.890.553.91661614303403040.160.850.493.5786201634406

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# 4815167

Several sample size calculation methods while controlling FDR have been proposed in microarray experiments. For example, Liu and Hwang [14] developed a method to calculate sample size given a desired power and a controlled level of FDR by finding the rejection region for the test procedure and hence power for each sample size. Hereafter, we call this sample size calculation method the LH method. Orr and Liu [15] assembled the ssize.fdr R package which implements the LH method.

The rejection region depends on the test applied for differential expression, and the method based on (1) can be applied to any multiple testing procedure where the same rejection region is used. This LH method can be implemented using an R package, ssize.fdr, developed by Orr and Liu [15], and applied for designing one-sample, two-sample, or multi-sample microarray experiments. The method would be applicable to RNA-seq experiments if we can calculate power and type I error rate given a rejection region.

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R49 was used to perform the data processing and analyses of mRNA data. Student’s t-test was used to compare expression levels between the patient and control groups. Power of the gene expression data was estimated using R package ssize.fdr50,51. Clustering heat maps were plotted with gplots52. Gene expression data were filtered using R package genefilter53. Gene ontology (GO) analysis was performed using R package piano54. KEGG pathway analysis was performed using R package GeneAnswers55,56, and GO pie chart was plotted using R packages geneListPie57. Protein-protein interaction (PPI) network was retrieved via STRING v9.158. The networks were plotted using Cytoscape59.

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# 4527836

A total of 9 BALB/c mice were used in the present study and were randomly allocated into three experimental groups. Six mice were each orally infected with a volume of 30 μL of PBS solution containing five F. hepatica metacercariae and served as experimental groups. Three additional mice were used as uninfected controls and were orally administered with a volume of 30 μL of PBS. The ssize.fdr package for R was used to estimate the sample size, taking into account the recommendations by Orr MP et al., 2009 [20].

# 5788701

The R package ssize.fdr4 was used to calculate the sample size needed for a microarray experiment powered to detect differentially expressed genes among patients who responded to treatment (responders) at week 16 (end of treatment).5 Estimates for sample size calculations were based on previous experiments with anti-TNF agents that used a similar study design.6 A sample size larger than 30 guaranteed that we could detect differentially expressed genes at a false discovery rate (FDR) of less than 0.05 and a fold change (FCH) of greater than 2.0 with a power higher than 80% for week 16.

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# 4441120

Using the R package ssize.fdr, power calculations were performed to determine the number of samples needed to detect differentially-expressed genes with a fold-change between treatments of as low as 1.3 with 80% power. Based on these power calculations, the experiment was designed to include six replicates of each condition. The order of sample processing was randomized with respect to treatment in order to avoid creating confounding batch effects.

# 4287952

Furthermore, we compared the power assessment results from PROPER and ssize.fdr, the R package based on method by Liu and Hwang (2007) for microarray data. In general, we found that ssize.fdr over-estimates power (Supplementary Section 7 and Supplementary Fig. S8). That is because ssize.fdr does not take into account the sequencing depth information, and assumes that the power of detecting DE genes only depends on the effect sizes. The comparison demonstrate that power calculation method developed for microarray data is not applicable for RNA-seq data and may lead to erroneous results.

# 3821217

As for other -omics approaches, undersampling (too low sample number) leads to overfitting. Simply put, statistical tables contain too many columns (endpoints) compared to rows (sample replicates). The choice of the right sample size is essential for a conclusion regarding whether a marker behaves differently from the controls or not. The sample size should be determined from preliminary experiments in which different sample replicates are set and the internal variability among samples is used to estimate the number of replicates to achieve statistically significant results. Statistical rules suggest that the sample size needs to be matched to the number of metabolites and to the required statistical power. For the estimation of the right sample size for metabolomics approaches, some in silico tools can be recruited, e.g., the programs nemaed samr, ssize, and ssize.fdr. Notably, the variables for these estimation tools have to be well chosen, e.g., number of measured metabolites and the relative abundance of the metabolite concentrations. In practice, it often will not be possible to adhere completely to the stringent rules of statistics. Compromises need to be found that still allow technical feasibility.

# 2958130

Microarray technology [1] has emerged in the last decade as the favoured method for large-scale gene expression studies. The technique can be used to simultaneously analyse the expression of thousands of genes and requires relatively small amounts of starting RNA material, therefore it provides a powerful tool for the comprehensive analysis of tissue or cell biology in response to a given stimulus such as; an infection [2], [3], a disease such as cancer [4]–[6], chemoresistance [7] or development, e.g. cell differentiation [8]. This means that the relationships between genes and their involvement in specific cellular functions can be better characterized. However, owing to the large number of genes and to the small number of samples, there are many statistical problems associated with microarray data [9], [10], which makes the detection of differential gene expression a challenging task. One of the main problems is the huge amount of data generated by microarray technology. Consequently, algorithms such as Ingenuity Pathway Analysis, LSGraph, Cognia Molecular, Metacore, or Bibliosphere were developed to analyse and understand complex biological systems. However, distinguishing genes that undergo expression variation (EV) among all the genes analysed remains difficult. Consequently, the normalization of gene expression data [11] and the development of methods to identify genes undergoing expression variation (EV) would represent an important step forward. A number of papers have described methods for assessing selected dataset requirements in microarray experiments using statistical criteria [12]. However, in all cases, the selection of genes undergoing expression variation is associated with a stringency parameter. Lee and Whitmore [13] used an ANOVA model and provided power calculations for various alternative models. Muller et al. [14] used a decision-theoretic approach and a hierarchical Bayes model. Wei et al. [15] examined the roles of technical and biological variability, in determining a selected data set. Pawitan et al. [16] assumed that genes are independent and have equal variance, and the paper reports on false discovery rates and sensitivities. Sample size calculations for a microarray experiment package (ssize.fdr package) [17] also assumed that the genes are independent, but pilot data is used to estimate the variance. It focused on test power and Type 1 errors (false negatives).