Data Request form YOUth (version 3.0, September 10, 2019)

Introduction

The information you provide here will be used by the YOUth Data Management Committee to evaluate your data request. Details on this evaluation procedure can be found in the Data Access Protocol.

Moreover, your data request will be stored in an online repository available to all researchers who submit or have submitted a data request. The aim of this repository is to provide a searchable overview of past, current, and pending data requests. By default, we will publish the following information from your request on our researcher's website:

- After submission of a data request: the names and institutions of the contact person and participating researchers (**Section 1**) and the research context (**Section 2**).
- After approval of a data request: the complete request (**Section 1-5**).
  Exception: If you believe that publishing the complete request could do harm (e.g. when you propose to use a novel analysis technique) you can object to publishing the complete request. This should be indicated on the data request form with a rationale (**Section 5**). The YOUth Data Management Committee will review your matter and advise the YOUth Executive Board whether or not to publish the complete request. If you do not agree with the YOUth Data Management Committee about publishing the complete request, you have the possibility to withdraw your data request.

Section 1: Researchers

In this section, please provide information about the researchers involved with this data request.

- Name, affiliation and contact information of the contact person
- Name and details of participating researchers (e.g. intended co-authors)
- Name and details of the contact person within YOUth

Contact person for the proposed study:	
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Section 2: Research context

In this section, please briefly describe the context for your research plans. This section should logically introduce the next section (hypotheses). As mentioned, please note that this section will be made publicly available on our researcher's website after submission of your request.
Please provide:

- The title of your research plan
- A very brief background for the topic of your research plan
- The rationale for and relevance of your specific research plan
- The specific research question(s) or aim(s) of your research (Please also provide a brief specification)
- A short description of the data you request

References can be added at the end of this section (optional).

Title of the study
Epigenetic traces of maternal alcohol use in children at birth

Background of the topic of your research plan, rationale, relevance (max. 500 words) 364 words
Mounting evidence suggests that perinatal environment is associated with susceptibility to adult disease risk(1). Maternal perinatal exposures impact on neurodevelopment and cognitive function in their offspring(2). Unraveling the underlying biological mechanism of these relations can provide measures for prevention, early intervention and the development of biomarkers, consequently reducing the risk on development of adult disease, psychiatric disorders and aberrant brain development(1,2). A putative mechanism of the relationship between maternal exposures and offspring brain development is changes in epigenetic regulation in the offspring. DNA methylation is one of the epigenetic mechanisms that plays an important role in cellular responses to environmental influences(3). Nutrition as maternal environmental factor may have an impact this way: recent studies from our group confirm that early life exposure to nutritional deprivation is associated with stable DNA methylation differences(4–6) and vulnerability to psychiatric disorders(7) in offspring later in life. However promising, further investigation of this underlying mechanism is needed for progression in means of prevention, early intervention and the

development of biomarkers. As a first step in investigating a putative chain exposure-epigenetics-altered brain development, alcohol is a key exposure. Alcohol is a teratogen causing damage to fetal brain and organ development, leading to fetal alcohol spectrum disorders (FASD) with fetal alcohol syndrome (FAS) being the most severe and visibly notable(8,9). Previous research shows distinct DNA methylation patterns associated with FASD and heavy alcohol consumption(10,11). However, in general population, most women who drink alcohol during pregnancy do so at light to moderate levels(12). In the YOUth cohort about 31 percent of the offspring is exposed in the first trimester(13). Considering that light to moderate alcohol consumption can also impact on cognition, behavior and gestational age(14,15), it is of interest to understand these mechanisms and develop biomarkers. The possible DNA methylation changes related to light and moderate alcohol consumption are therefore of interest.

Considering the documented link between maternal alcohol use, epigenetic changes and development of the brain of the offspring, we hypothesize that maternal alcohol consumption during pregnancy leads to differentially methylated regions (DMRs) in offspring DNA and subsequently impacts on offspring brain development. The YOUth cohort has obtained unique data to answer this question with the availability of data on maternal nutrition, on the development of the brain and cognition in the offspring and of offspring's cord blood for epigenetic measurements.

The specific research question(s) or aim(s) of your research

Do epigenetic changes mediate a relationship between maternal alcohol use and altered fetal brain development?

Summary of the data requested for your project: Please indicate which data you request to answer your research question.

We would like to receive the following data on 192 mother-offspring pairs from the YOUth baby&child cohort:

Mothers (N=192)
- Demographics, periconceptual and general health
- Lifestyle questionnaires
- Diet and quantity of alcohol consumption using the Food Frequency Questionnaire (FFQ)(16,17) that will be also used to assess macronutrients (carbohydrates, protein (animal/vegetal), fats), caloric intake and dietary style as possible confounders as alcohol use may be related to dietary intake that in term is related to DNA methylation (6)
- Brief Symptom Inventory (BSI) to assess maternal mood as a confounding factor(18)
- Childhood Trauma Questionnaire (CTQ) to assess childhood trauma as a confounding factor (19)

Offspring (N=192)
- APGAR score and delivery information (parent report)
- 3D-echo at 30 weeks (N=192): Advanced neurosonography is used to study the fetal brain development and includes reliable measures of occipitofrontal diameter, intracranial volume, transcerebellar diameter, cerebellar volume, and thalamic width, area, and volume (20). In 150 participants 3D echo data is already preprocessed and data on an aggregated level and complete data at two time points is available in 120 participants. From the remainder required 62 participants the raw data 3D echo data is available and will be preprocessed by Marieke Albers as part of ongoing research efforts.
- Development at 5 months and 10 months including child health and growth (obtained from parent questionnaires)

- Chord DNA whole genome DNA methylation profiling using illumine EPIC arrays with 850,000+ loci
- Genotypes

Biomaterials:
The best possible reflection of intra-uterine environment can be obtained from epigenetic analysis of cord blood since placental material is not available in this study. Cord blood was taken at birth and send to the Child Expertise Center by regular mail. Cell type composition of cord blood can be estimated using reference data and available scripts (21,22), and recent developments provided means for analyzing the association of methylation with maternal exposures per cell type (23). Methylation of DNA is very stable (24), but differences in integrity of the DNA between samples may result in technical variations and non-random sample failure. Because of the less than optimal collection of the chord DNA (personal communication) for epigenetic measurements we will first test DNA quality before additional analysis are performed.

References (optional)

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Section 3: Hypotheses

In this section, please provide your research hypotheses. For each hypothesis:

- Be as specific as possible
- Provide the anticipated outcomes for accepting and/or rejecting a hypothesis (or explain why this does not apply to your project, e.g. when using Bayesian statistics)

Exception: if you plan a hypotheses-free project, please use this section to explain why you don't formulate specific hypotheses.

Hypotheses
Differentially methylated regions (DMRs) in the offspring that are related to maternal alcohol use are determinants of offspring brain development.

Section 4: Methods

In this section, you should make clear how the hypotheses are tested. Be as specific as possible. Please describe:

- The study design and study population (Which data do you require from which subjects?)
- The general processing steps (to prepare the data for analysis)
- The analysis steps (How are the data analysed to address the hypotheses? If possible, link each description to a specific hypothesis)
- Any additional aspects that need to be described to clarify the methodological approach (optional)

Study design, study population and sample size (e.g. cross-sectional or longitudinal; entire population or a subset; substantiate your choices)

Study population:
192 mother-offspring pairs from the YOUth baby&child cohort, from 20 weeks of pregnancy to 10 month old newborns.

Main determinants:
Considering the wealth of phenotypes that are available in this study we will need to prioritize. Focus will be on alcohol use in the first trimester that was present in about 31% of the mothers(13). Alcohol use will be quantified in amount of units following the Food Frequency Questionnaire (16,17). Macronutrients, caloric intake, smoking, mood, childhood trauma and dietary style will be assessed as potential confounding factors.

Development:
Analysis of fetal brain development will be conducted using 3D-ultrasound imaging. Also, analysis of neurodevelopment will be conducted using the identified epigenetic marks (DMP, DMR and networks) as indicators of brain developmental parameters.

Power estimate:
Whereas genome-wide association studies (GWASs) aim to identify genetic variants (absent or present) associated to common diseases, epigenome-wide association studies (EWASs) examine associations of epigenetic differences associated with traits, taking both genetic and environmental factors into account(25). DNA methylation marks are involved in gene expression regulation and have been extensively studied for complex diseases(25). Epigenetic analysis of DNA methylation studies uses genomic information on a continuous scale (percentage of methylation in loci). Regional differences in DNA methylation (DMRs; differentially methylated regions) consist of several of such marks and are biologically relevant as they can provide gene promotor hypermethylation with subsequent impact on the activity of genes. Power is therefore superior for EWAS than for GWAS, particularly for analysis of DMRs.

For power estimation we used effect sizes derived from a recent meta-analysis of population studies on maternal alcohol consumption and offspring DNA. This study identified 9 DMRs associated with first trimester drinking (multiple testing corrected p-value $<1 \times 10^{-6}$) and reported effect sizes ranging from d=0.56-1.63 for the most and least associated DMRs respectively(26, supplementary tables, table S14). A sample size of 192 as here proposed will have 90 percent power to detect effect sizes of 0.52, sufficient to also detect the smallest DMR found in this meta-analysis.

No effect sizes are known on the relation of DNA methylation patterns and brain volumes to our knowledge. However, the relation between prenatal alcohol exposure and reduced brain volume is commonly known, has been extensively studied and was reported in samples as small as 10 (27).

General processing steps to prepare the data for analysis

Quality control (QC) and genomic analysis:
QC of genetic and DNA methylation data is performed using a standardized pipeline operational in our group for several years now and has been described extensively previously (6,28,29). In short for the methylation data we use the on-array control probes and the technical variations that we encounter to functionally normalize the methylation data using surrogate variables derived from these factors in order to obtain unbiased normalized DNA methylation levels(30). All analysis will be conducted using the R package for statistical computing. Genotypes will be processed using standard genotyping pipeline implemented in PLINK and subsequently imputed using Impute.

Processing of fetal 3D-ultrasound:
Advanced neurosonography is used to study the fetal brain development and includes reliable measures of occipitofrontal diameter, intracranial volume, transcerebellar diameter, cerebellar volume, and thalamic width, area, and volume(20). These data will be processed in collaboration with Roel de Heus and Marieke Albers as previously.

Additional methodological aspects (optional)

Specific processing and analysis steps

Primary analysis will be of the relation of maternal nutrition with DNA methylation profile in the offspring after quality control using multivariate linear regression analysis. Both differential methylation positions (DMPs) as well as differentially methylated regions (DMRs) will be identified. Secondary analysis will include genetic modification of this relation based on polygenic risk scores (PRS) of related traits (PTSD, depression and educational performance) as well as modifications by genetic variation of the offspring in the same genetic region (100bp). Data reduction of the DNA methylation data will be done using Weighted gene co-expression analysis (WGCNA)(31) to derive networks of genes and their relation to nutrition. As additional analysis epigenetic age will be estimated using the Horvath and Levine age predictors to investigate deviations from the predictors in relation to nutrition (32,33). Comprehensive analysis of potential confounding and sensitivity analysis will be conducted.

Section 5: Data request

In this section, please specify as detailed as possible which data (and from which subjects) you request. Include information regarding:

- Which wave(s)
- Which experiments, questionnaires, etc.
- How many sets (sample-size)
- Purpose of your data request
- Other aspects relevant to your data request (optional).

Select the appropriate wave(s) (more options are possible):

☒ Rondom zw – 20 weeks
☒ Rondom zw – 30 weeks

- ☒ Rondom 0 – 5 mo
- ☒ Rondom 0 – 10 mo
- ☐ Rondom 3 (not available yet)
- ☐ Rondom 6 (not available yet)
- ☐ Rondom 9
- ☐ Rondom 12 (not available yet)
- ☐ Rondom 15 (not available yet)

Experiments and number of sets you request
We would like to receive data on 192 mother-offspring pairs from the YOUth baby&child cohort: see attached excel file 'DataSelectionTemplate v1.0'.

Other aspects relevant to your data request (optional)

Data request for the purpose of:

- ☒ Analyses in order to publish:
  - ☒ Article
  - ☐ Report
  - ☐ Thesis
  - ☒ Other. Please specify Applications for further funding
- ☐ Analyses for data quality control only (data will not be published)
- ☐ Analyses for descriptive data only, e.g. in order to determine good datasets (data will not be published

DISCLAIMER DATA ACCESS QUALITY CONTROL AND DESCRIPTIVE DATA: These data can only be used for data quality control analyses or descriptive data analyses only and may not be made public, for example by publishing them or otherwise making them available to others. If you want to use data for disclosure, permission of the YOUth data committee is required, and this data request protocol must be followed for analyses in order to publish.

Would you like to be notified when a new data lock is available?
In principle, data will be made available in data locks twice a year. This means that twice a year, the data is locked on a specific date and that all approved data request projects will receive the same locked data set.

- ☒ Yes
- ☐ No

Do you agree with publishing the complete request on our researcher's website after it is approved (by default)?

- ☒ Yes
- ☐ No. Please provide a rationale below.

NOTE 1: Please fill out the 'Form contributions to YOUth data collection' in Annex 1 to specify your contribution to YOUth in order to gain access to the requested data.
NOTE 2: Please fill out the 'Data Selection Template' (.xlsm) to specify the sort of data you request.

This Annex 1 together with the Data Selection Template and this Request Form should be sent to the Secretary of the Scientific Director (I.Bleeker@uu.nl).