Title of the study (one request per article):
Longitudinal examination of fetal and neonatal brain maturation in typically developing infants and preterm born neonates.

Contact person for the proposed study: (please note that this should be level postdoc or higher)	
Name:	M.J.N.L. Benders (PI fetal and neonatal MRI) and Elise Turk (PhD student on project)
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Wave (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)

We ask you to provide us with a clear background, methods section and data-analysis plan. These parts of the proposal will be publicly displayed for reference.

Background of the project (max. 500 words): Please provide a short background including the rational of your study as you would do in an introduction of the paper
The third trimester of pregnancy and the neonatal period is characterized by an immense expansion of the cortical surface area, the cerebellum and developing white matter tissue, caused by the migration of neurons and axonal growth and myelination. The cerebellum increases in 3.5-fold (Volpe, 2009b) and the frontal, temporal, parietal and occipital lobe gradually undergo secondary and tertiary foliation, resulting into gyrification that is typical for the human brain by the time of birth (Striedter et al., 2015). Given the quantity and complexity of brain changes during third

trimester development, this period is particularly vulnerable for potential insults including extra-uterine exposure which is unique to preterm born infants.

The preterm brain is particularly vulnerable to early developmental insults that are common in preterm birth, including white matter or cerebellar injury and strokes (Ortinau & Neil, 2015; Stoodley & Limperopoulos, 2016; Volpe, 2009a). Available evidence suggests that premature exposure to the extra-uterine environments disrupts the normal developmental course of the brain, even in the absence of direct brain injury. Alterations in the structural and functional connectome, decreased white matter volume and cerebellar growth impairment are increasingly detected in survivors of prematurity (de Kieviet et al., 2012; Brossard-Racine et al., 2017a). Additionally, longitudinal studies show that preterm born neonates are at greater risk of developing neurodevelopmental deficits and psychiatric disorders than full-term born neonates, even without evidence of macroscopic brain injuries (Ortinau & Neil, 2015; Volpe, 2009a).

Although the preterm neonatal brain has been studied quite intensively, MRI studies of typical in utero development and term born infants are limited and therefore comparisons between in and ex-utero development are lacking from literature. Recent advantages in fetal and neonatal MRI have made it possible to obtain normative information about brain function or functional networks (e.g. Thomason et al. 2015; van den Heuvel et al. 2015) and structural brain growth (Gholipour et al. 2017). A pioneering study of Bouyssi-Kobar et al. (2016) shows global volumetric differences of the brain between healthy fetal and preterm infants using MR images. A longitudinal MRI study of Brossard-Racine et al. (2018) demonstrated reduced cerebellar growth in very preterm neonates compared to healthy fetuses and neonates during the third trimester.

Previous studies did not evaluate longitudinal brain growth or functional connectivity of multiple regions in healthy and preterm born infants using MRI, which could help elucidate selective vulnerability for later functional deficits. Therefore, in the current study, we aim to describe brain development between 29 and 46 weeks of gestation and sought to characterize functional and structural differences of in and ex-utero brain development.

Research question

Our aim is to explore structural and functional brain development between 29 and 46 weeks of gestation. By mapping both typical fetal and neonatal as well as preterm neonatal brain maturation, this study will provide important insights of brain development and shed light on differences between preterm and term born neonates. We hypothesize that early extra-uterine exposure alters regional brain growth and function in infants born preterm.

Methods Describe the methods as in the paper in which the data will be presented, according to the categories below, with a total **maximum** of 1500 words. For a description of task, methods etc. refer to the website, if possible.

Design of the study (for instance cross-sectional, longitudinal etc.; substantiate your choices)

Our study aims to study longitudinal brain development in the last trimester of pregnancy and during the first month after birth, additionally, we want to compare brain development of two populations: typically developing intrauterine brain of full-term born infants of the YOUth cohort and preterm born neonates without major brain deficits (WKZ cohort) in the equivalent period according to corrected age.

Both cohorts are longitudinally scanned on a 3 Tesla Philips system within the UMC Utrecht.

Normal developing infants are scanned twice; first in utero between 30 and 34 weeks GA and secondly within a month after birth. The typically developing infants are part of the YOUth cohort. Further description of the study design is described on the YOUth website: https://youthonderzoek.nl/baby-kind/mri-onderzoek-bij-babys/

The longitudinal MRI data of the preterm neonates is obtained around 30 weeks of gestation and a second time around 40 weeks of gestation as standard clinical care. The data will be selected retrospectively (nWMO) **when no macroscopic brain injury is visible.**

Study population and sample-size (entire population or a subset; substantiate your choices e.g. Provide a rationale for the requested sample-size, for instance using a power calculation)

All MR images of typically developing full-term born babies will be included from the YOUth baby cohort. An estimated n=27 +/-3 fetal scans and 14 +/-3 neonatal scans from the YOUth cohort will be included in this study. We are aiming to select those infants without abnormal neurodevelopmental outcome. If there will any concern according to abnormal development these children will be excluded. The data of an estimated N=200 preterm born babies will be selected retrospectively from a total of ~N=600 participants (WKZ cohort), who will have a neurodevelopmental outcome at the age of 2 years with normal range (Bayley assessment).

MRI-research with healthy full-term born perinates is sparse. Most examples of healthy fetal and neonatal MRI can be found in clinical trial studies, in which fetuses of 24 to 194 healthy pregnant volunteers (e.g. (Brossard-Racine et al., 2014; Schellen et al., 2015)) and between 16 and 20 neonates, without abnormalities on the MRI scan (e.g. (Ball et al., 2014; Bertholdt et al., 2013; Miller et al., 2007)), are scanned as a control group. These studies found differences between patients and healthy controls in brain volume, cortical connectivity, diffusivity, fractional anisotropy, brain organization and neurological abnormalities. Scanning in these age ranges is difficult to acquire and when obtained therefore an unique population. According to literature we believe that we can make a detailed description of the primary fetal and neonatal outcomes with a small normal cohort (see for example, (Lefèvre et al., 2015; Pandit et al., 2014; Thomason et al.,

2014)). It will be unique to also have longterm outcome in these infants, so being informed to have a normal population.

Data processing and preparation (including necessary recoding of data etc.)

Fetal and neonatal T2-weighted MR images will be segmented and parcellated to determine brain volumes, cortical surface area and gyrification. Additionally, we will examine the developing functional connectome by the resting state brain activity acquired using fMRI.

Volumes of different brain areas will be determined by an in-house developed semi-automatic brain masking, after motion correction and segmentation pipeline (Moeskops *et al.* 2016; Khalili *et al.* 2017; 2019a; 2019b). Resting state brain connectivity will be determined using functional MRI using FSL and SPM (see for example (van den Heuvel *et al.* 2018; Turk *et al.*, provisionally accepted by Journal of Neuroscience))

Handling missing data (describe how you will detect and handle missingness in the data)

Within the YOUth cohort, motion, inhomogeneity or discontinuing of the scan due to discomfort can be a reason to exclude the data of one of the two scans. Due to exclusion of or withdrawal from the participants it is also possible that the second scan will not take place. Since both scans are valuable on itself, the remaining data of the concerned participant will still be included. The preterm data will be selected prospectively. We will analyse the data separately per (corrected) gestational age group, around 30-34 wks and 40-43 wks. Additionally we will analyse brain development and growth by using the longitudinal data of those infants with available data. This will be an exploratory approach since the amount of patients.

Data analysis methods (including statistical design and statistical analysis plan. If it is not possible to provide a detailed statistical plan, as this does not fit in with the research questions formulated above, please explain.)

Using convolutional networks, we will perform global segmentation of T2 weighted images into seven classes: cerebellum, (un)myelinated white matter, deep gray matter, ventricles, brainstem, cortical gray matter, and cerebrospinal fluid. Automatically segmented images will be reviewed and corrected manually in Image Explorer if necessary (Khalili *et al.*, 2017;2019a;2019b). Brain volumes and cortical folding and surface measures can be computed by in-house developed algorithms.

Further parcellation of segmented areas will be performed using manual annotations in Freesurfer and TKsurfer of the T2-image or age-matched template brain (Ghlipour et al. 2017). The processed T2 images are further used as an atlas for the reconstruction of resting state connectivity (van den

Heuvel *et al.*, 2018; Turk *et al.* provisionally accepted by Journal of Neuroscience). Resting state connectivity will be determined using functional MRI. Network analysis will be performed to quantify key features of the functional connectome (modules, small-worldness, clustering, node centrality).

To quantify brain growth between 29 and 46 weeks of gestation and to explore differences in MRI parameters (global, and regional volumes, cortical folding, and functional connectivity) between YOUth infants and preterm neonates (after correction for gestational age), we will perform a multiple linear regression/ANCOVA for all subjects.

Planned subgroup analyses (if applicable. Substantiate your choices)

The main analysis will be to quantify brain development and growth over time (first and second scan) between preterm and full term born neonates. As a sub analysis the two cohorts will be compared. To take GA into account we perform a multiple linear regression/ANCOVA.

Planned sensitivity analyses (if applicable. Substantiate your choices)

Sensitivity analyses are analyses that you plan beforehand to test whether certain factors have a major influence on your results.

The data of the preterm neonates and full term fetuses and neonates are scanned using different scanning-protocols. Image intensities will differ between fetal and neonatal scans due to differences in maternal/external environment. It is important to test if the scanning protocol differences have an effect on the data.

Due to movement during scanning the images can contain inhomogeneity. Strong in-house techniques are developed to correct for this (Khalili *et al.*, 2019b).

In our prior studies, we show the robustness of neonatal scan segmentation (Moeskops *et al.* 2016). We will visually check if the different orientations lead to consistent results among the different brain volumes.

2. **Timeline and milestones (including dates of when to analyze/write up):**
   The current preliminary study will last 8 months. An estimation of a timetable:
   Aims month 1: Literature study, make study design, select methods and techniques (finished).
   Aims month 1-3: Start data preprocessing and analysis, immediately after approval of using the data. Finish article introduction and method section.
   Aims month 4-5: Data analysis, analyze results, discuss and reflect on work. Write result section.
   Aims month 6-8: Finishing paper/chapter in PhD thesis.

3. **Output (e.g. article, report, etc.):**
   Article: Exploring structural and functional brain development of preterm infants ex utero to typically developing fetuses in utero during the third trimester of pregnancy and first month of life. This manuscript is ment to be a chapter of PhD thesis of Elise Turk.

4. **Proposed authors + affiliations (please note that the YOUth data access committee can request certain authors to be included):**
   - Turk, E., department of Neonatology and Obstetrics, UMC Utrecht. PhD student and coordinator of Baby MRI within YOUth.
   - Benders, M., department of Neonatology, UMC Utrecht. PI on project
   - de Heus, R., department of Obstetrics, UMC Utrecht
   - Franx, A., department of Obstetrics, UMC Utrecht
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   - van den Heuvel, M.P., department of Genetics, VU Amsterdam
   - Kahn, R.S., Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York
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This form should be sent to: Secretary of Chantal Kemner: i.bleeker@uu.nl

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