ON-LINE APPENDIX

MR Imaging Methods Sensitive to Infant Brain Development and Used in This Study

Diffusion tensor imaging Tract-Based Spatial Statistics, an objective MR imaging method to evaluate brain white matter microstructures, can sensitively detect changes in normal white matter development—that is, illustrating the association between maternal obesity at early pregnancy in otherwise healthy women and lower white matter integrity in their full-term healthy neonates.[1] Likewise, resting-state function MR imaging, a functional neuroimaging technique that does not require task-based stimuli and is, therefore, ideal to study brain functioning in young infants, was able to show decreased brain functional connectivity associated with maternal obesity in healthy neonates.[2] Myelin water fraction imaging can provide an additional MR imaging measure that reflects both tissue microstructure (ie, myelin content) and brain connectivity and has revealed some of the earliest brain changes in infants (eg, those associated with genetic predisposition to Alzheimer disease).[3] Furthermore, these MR imaging methods can also sensitively detect brain developmental differences in older healthy children that are consistent with their neurodevelopment. For example, both DTI and MWF measurements have shown better white matter development associated with breastfeeding in healthy children[4,5] that positively correlated with IQ scores.[5] In addition, other MR imaging methods such as voxel-based morphometry and tasked-related fMRI can detect small differences (eg, those associated with infant diets) in brain gray matter structure and function in healthy children, consistent with differences observed in performance for language and perception tasks.[6]

Study Population: Detail Information

Cohort 1. Subjects were 2-week-old full-term healthy appropriate for gestational age neonates from a longitudinal observational study of the mother's BMI at early pregnancy and offspring's growth and development (ClinicalTrials.gov NCT01131117, infants with valid clinical and MR imaging data at 2 weeks of age were included, $n = 43$). Inclusion criteria for the recruited women were second parity singleton pregnancy (<12 weeks of gestation), conceived without assisted fertility treatment, and 21 years of age or older. Exclusion criteria for the women were pre-existing medical conditions such as diabetes mellitus, hypertension, seizure disorder, psychiatric disorders, or sexually transmitted diseases; medical conditions and complications developed during pregnancy such as gestational diabetes and preeclampsia; medications during pregnancy that may influence fetal growth; and smoking or alcohol consumption during pregnancy. Inclusion criteria for the infants were healthy and term at birth (≥37 weeks of gestation). Exclusion criteria for the infants were medical conditions or medications known to influence growth and development. All mothers had their body composition assessed using air displacement plethysmography (BOD POD; COSMED, Chicago, Illinois) before or within the first 12 weeks of pregnancy. Maternal IQ was assessed using the Wechsler Abbreviated Scale of Intelligence (WASI; Pearson, San Antonio, Texas) for all except 1 subject. Height and weight were measured at 12 weeks of pregnancy, and BMI was calculated. Gestational weight gain was measured at 36 weeks of pregnancy (compared with the weight at 12 weeks). Infant birth weight and length were obtained at delivery. At 2 weeks of age, head circumference was measured, infant diet information was documented (specifically, those with exclusive breastfeeding for 2 weeks or mixed feeding including breast milk were regarded as breastfed; those with exclusive formula feeding were regarded as formula-fed).

Cohort 2. Subjects were healthy 7.5- to 8.5-year-old children from an observational study of how infant diet during the first year of life affects brain development in children (ClinicalTrials.gov ID: NCT00735423, all children with delivery-mode information available and valid MR imaging data were included). All children were healthy, with no maternal use of psychotropic medications or alcohol, tobacco, or drug issues during pregnancy. All children had no history of psychological or psychiatric diagnoses, neurologic impairment or injury, use of anticonvulsant, stimulant, or mood-stabilizing medications; sleep disorder; or other serious illness or diseases. All children were either predominately breastfed (exclusive breastfeeding for at least first 4 months and continued breastfeeding throughout the first year) or formula-fed (on formula for most of the first year). IQ was tested by the Reynolds Intellectual Assessment Scale, language skills were measured by the Clinical Evaluation of Language Fundamentals, and memory index was measured by the Children's Memory Scale.

Cohort 3. Subjects were healthy, typically developing, 3- to 60-month-old children, a community sample recruited from Providence, Rhode Island, and surrounding areas. To maintain a focus on healthy and neurotypical development, we excluded children with major risk factors for intellectual, behavioral, or other developmental disorders. Selection criteria were healthy singleton birth between 37 and 42 weeks of gestation; no abnormalities on fetal ultrasonography; no reported history of neurologic events or disorders; no admissions to the neonatal intensive care unit; no family history of a psychiatric or neurologic disorder in the parents or siblings; and no complications (eg, preeclampsia) or reports of illicit drug or alcohol use during pregnancy. These criteria were confirmed during parental interviews at the time of enrollment. Extensive child, parent, and sibling medical and family history questionnaires were used to provide information about the subject's neurologic, psychiatric, and other medical events and disorders (if any) and maternal prenatal and postnatal health.

MR Imaging Data Acquisition: Detail Information

The MR imaging data for cohorts 1 and 2 were acquired on a 1.5T Achieva MR imaging scanner (Philips Healthcare, Best, the Netherlands) with an 8-channel sensitivity encoding head coil at the Arkansas Children's Hospital. For cohort 1, the 2-week-old neonates were scanned during natural sleep without sedation. They were fed ~30 minutes before the scan, swaddled in warm sheets, and wrapped by a MedVac infant immobilizer (CFI Medical Solutions, Fenton, Michigan). A pulse oximeter probe (InVivo, Gainesville, Florida) was placed on a foot to monitor oxygen saturation and heart rate, and Mini-Muffs were placed over the ears to block noise from the scanner. An MR imaging–compatible camera was attached to the head coil to monitor the infants. Regular MR imaging, including diffusion, susceptibility, and 3D T1-weighted imaging, was used to exclude apparent brain abnormal-

ities. A single-shot spin-echo EPI sequence with diffusion-weighting gradients in 15 uniformly distributed directions and a maximum b-value of 700 s/mm^2 was used to acquire DTI data. Other parameters were the following: shortest TR = ~4200 ms, TE = 66 ms, FOV = 180 × 180 mm, voxel size = 2 × 2 mm with 3-mm slice thickness, 30–36 contiguous axial slices, 4 averages, and reconstruction matrix size = 128 × 128. In addition, a single-shot gradient-echo T2*-weighted EPI sequence with TR/TE = 2400/50 ms, acquisition voxel size = 2 × 2 × 4 mm^3, and 150 dynamics was used to acquire RS- fMRI data. Quality control was performed to exclude subjects with excessive motion during the scan. Specifically, for DTI, motion was evaluated by inspection of the scanner-generated color fractional anisotropy maps and head movement/imaging artifacts in raw images. For RS-fMRI, motion was initially evaluated by scrolling through different dynamics of the raw images for large translation or rotation of the brain and further regressed in the postprocessing (see data analysis section for details).

For cohort 2, the 8-year-old children were scanned without sedation. A single-shot spin-echo EPI sequence with diffusion-weighting gradients in 15 uniformly distributed directions and a maximum b-value of 800 s/mm^2 was used to acquire DTI data. Other parameters were the following: TR = 5135 ms; TE = 66 ms; FOV = 220 × 220 mm; voxel size = 2 × 2 × 3 mm^3; 34 contiguous axial slices; 2 averages; and reconstruction matrix size = 128 × 128. In addition, a sagittal T1-weighted 3D turbo field echo sequence with TR = 7.3 ms; TE = 3.4 ms; flip angle = 8°; acquisition voxel size = 1 × 1 × 1 mm^3; matrix size = 256 × 232 × 150; and 2 averages was used to acquire high-resolution structural imaging data for VBM analysis. Quality control was performed on the basis of screening of raw images to exclude subjects with apparent motion artifacts.

For cohort 3, longitudinal MR imaging data were acquired on a Tim Trio 3T scanner (Siemens, Erlangen, Germany) equipped with a 12-channel head radiofrequency array coil. All imaging was performed at night during natural sleep. Scan times were kept as short as possible, and noise levels were reduced by slowing the gradient switching rate and lowering the maximum gradient amplitude. Further acoustic attenuation was achieved to reduce the likelihood of the child waking during the scan through a Quiet Barrier sound-attenuating bore insert (Ultra Barrier HD Composite; Quiet Barrier, Chambersburg, PA) and electrodynamic headphones that played white noise throughout the acquisition. Appropriately sized pediatric MedVac bags and foam cushions were also used to cradle the infant and limit intrascan motion. MWF data were acquired using mcDESPOT (multi-component driven equilibrium single pulse observation of T1/T2) imaging protocols optimized for infants and young toddlers.[7] These protocols included 8 T1-weighted echo-spoiled gradient-echo images, 2 inversion-prepared echo-spoiled gradient-echo images, and 16 T1/T2-weighted steady-state free precession images. Imaging protocols were developed to maintain a constant voxel resolution of 1.7 × 1.7 × 1.7 mm, while the FOV and other parameters were varied to accommodate changing head size and noise tolerance. All acquired data were visually inspected to ensure that they were free of motion or other corrupting artifacts. Longitudinal MWF data were acquired at approximate 6- and 12-month increments (children younger than 2 years of age were scanned biannually; children older than 2 years of age, annually).

MR Imaging Data Analysis: Detail Information

Cohorts 1 and 2. Regular MR images were reviewed on the scanner and/or the PACS to screen for apparent brain abnormalities. DTI, RS-fMRI, and VBM raw data were exported to workstations with various imaging-postprocessing software for data analysis, as described below.

DTI. TBSS methods were used for DTI data analysis. Details were described in previous publications as to data for infants[1,8] and older children.[5,9] Briefly, the DTI parameter maps were computed and processed on a workstation with the FMRIB Software Library (FSL; http://www.fmrib.ox.ac.uk/fsl) installed on a VMware Linux virtual machine (VMware, Palo Alto, California). The generated fractional anisotropy (a main DTI parameter sensitive to white matter integrity) maps were preprocessed and aligned to each other to identify the most representative one, which consequently served as the target for nonlinear registration. The FA maps were skeletonized by the FSL TBSS program to create a mean template that included major white matter tracts (FA > 0.1 for neonates and FA > 0.2 for 8-year-old children), and all data were projected to the template for further analysis. The randomization program in FSL was used to perform voxelwise comparisons of FA values in the brain between subjects born by cesarean or vaginal delivery in each cohort. In addition, white matter ROIs showing group differences on TBSS analysis were sketched on the basis of anatomy using Matlab software (MathWorks, Natick, Massachusetts), and the mean FA values for each ROI were compared between groups.

RS-fMRI. Independent component analysis by the MELODIC toolbox in FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/MELODIC) was used to identify resting-state functional networks in the brain, and dual regression by FSL was used to compare the functional connectivity strength between infants born by cesarean or vaginal delivery. First, motion correction and denoise by FSL commands were performed, and subjects with rotation of >1.5° or translation of >1.5 mm in any direction on the processed images were further excluded. The MCFLIRT function in FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/MCFLIRT) was also used for additional motion correction of the imaging data. Brain images were extracted by the FSL Brain Extraction Tool (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BET) and were spatially smoothed with a 2-mm full width at half maximum. High-pass temporal filtering was used to remove low-frequency drifting artifacts. After these preprocessing steps, the RS-fMRI data for each subject were registered to a customized neonatal brain template generated on the basis of T1-weighted 3D brain data. Resampling resolution was set at 3 mm. The multisession temporal concatenation option in MELODIC with automatic dimensionality estimation was used to compute the independent components at a group level for all infants (and for each delivery mode group, respectively). The independent components computed by MELODIC were visually inspected to label meaningful functional connectivity networks based on anatomic locations of known networks in neonates[10-12] and exclusion criteria such as activation predominantly in the pe-

ripheral regions of the brain, in the ventricles, near major blood vessels (eg, circle of Willis), surrounded by ring-shaped deactivation, or in spotty patterns.[13] Dual regression and randomization tools in FSL were then used for voxelwise comparisons of the functional connectivity (z scores) in meaningful components between infants born by cesarean or vaginal delivery. The z score maps for each subject were also exported to Matlab for further ROI analyses.

VBM. High-resolution T1-weighted 3D structural images were exported to a workstation with Statistical Parametric Mapping software (SPM8; (http://www.fil.ion.ucl.ac.uk/spm/software/spm12). Methods are described in detail in previous publications.[6,9] Briefly, an age-appropriate pediatric brain template and customized tissue probability maps were created, and the raw images were segmented into brain white/gray matter and CSF. Nonlinear modulated normalized gray matter images were generated, and relative differences in regional gray matter volume corrected for individual brain size were compared between children born by cesarean and vaginal delivery.

Cohort 3: MWF. Following acquisition, the 26 individual echo-spoiled gradient-echo, inversion-prepared echo-spoiled gradient-echo, and balanced steady-state free precession images for each child were linearly coregistered to account for subtle intrascan head motion, and nonparenchymal signal was removed using an automated deformable model approach. Main magnetic field (B_0) and flip angle (B_1) calibration maps were calculated, and the MWF was estimated at each image voxel by fitting the data to a 3-pool multicomponent tissue-relaxation model.[7] The calculated MWF map was then nonlinearly aligned to a common analysis space in approximate Montreal Neurological Institute space. To help avoid potential bias in the registration of each child's longitudinal data, we took a stepwise approach in which a template was first created for each child to which all MWF maps were coregistered. The child-specific template was then coregistered to the study template, and this registration matrix was applied to the child's MWF maps. Using the aligned data, we obtained mean MWF values for 6 white matter regions, including frontal, temporal, parietal, occipital, and cerebellar white matter as well as the body of the corpus callosum. Nonlinear mixed-effects modeling was used to fit a modified Gompertz growth model to the longitudinal group-wise vaginal and cesarean delivery data, which were then compared between the delivery mode groups.

Statistics: Detail Information

For the comparison of demographic/anthropometric/neuropsychological data between delivery mode groups in cohorts 1 and 2, Wilcoxon rank sum tests were used for numeric parameters and Fisher exact tests (or χ^2 tests) were used for categoric variables. For the voxelwise comparison of DTI parameters in Tract-Based Spatial Statistics analysis and RS-fMRI parameters in dual-regression analysis, randomization with 5000 permutations was used with the threshold-free cluster enhancement option (testing of larger amounts of permutations did not change the results). To correct for multiple comparisons for the voxelwise analysis, we compared the observed TFCE image with the empiric null distribution computed across permutations of the maximum voxels-specific TFCE scores.[14,15] $P < .05$ corrected for multiple comparisons (voxelwise) was regarded as significant. Because multiple ICA components were defined as meaningful functional networks and fed into the dual-regression program for group comparison in the RS-fMRI analyses, an additional threshold of cluster size of >5 imaging voxels was used. For post hoc ROI analyses of DTI and RS-fMRI parameters, independent t tests were used after confirming data normal distribution and testing for equality of variance, and general linear model univariate analyses were used to compare differences with covariates controlled. For the VBM analyses of regional gray matter volume, 2-sample t tests with unequal variance were performed with multiple comparison correction to control for family-wise error. $P < .05$ family-wise error–corrected was regarded as significant. For cohort 3, the Gompertz function parameters for the MWF curve of the 6 regions were compared between delivery groups using nonparametric tests, with significance defined as $P < .0015$.

Potential confounders were controlled as covariates in statistical analyses. Specifically, for cohort 1, the TBSS and RS-fMRI dual-regression analyses were performed, respectively, when no potential confounders were added as covariates; when adding demographic parameters that were consistently different between groups ($P \leq .05$ for both the TBSS and the RS-fMRI subsets) as covariates (gestational weight gain, infant sex, and birth length); and when adding an additional 2 parameters that were known potential confounders identified by our previous studies (gestational age at MR imaging and maternal BMI at early pregnancy[1,2,16]) as covariates. For the post hoc ROI comparisons, significances with and without controlling for all of these 5 covariates were both evaluated. For cohort 2, the TBSS and VBM analyses were performed without adding covariates and then were repeated with age, sex,[17] and infant diet[6] added as covariates. Socioeconomic variables were not included as covariates because of incomplete data (group comparison on the available data did not show group differences other than mother's income).

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