Microstructure of the Dorsal Anterior Cingulum Bundle in Very Preterm Neonates Predicts the 'Preterm Behavioral Phenotype' at 5 Years

Supplemental Information

Cohort Retention

There are 94 VPT infants in this study, 83 of which had diffusion data. Of these 83 infants with diffusion data, 69 had usable cingulum bundle tractography after excluding for motion, poor quality scans, and other factors. At age 5 years, 80 out of the original 94 VPT infants returned for follow-up, giving a retention rate of 85% with 13 infants lost to follow-up (14%). Of these 80 children who returned at age 5 years, 56 infants of the infants had usable cingulum bundle tractography. The 13 infants were lost to follow-up because of they had outdated contact information and were unable to be traced or they failed to show up to booked assessment. There were no differences in age at scan (p=.496), medical risk at birth (p=.150), sex (p=.686), or race (p=.202) between the group that was lost to follow up and the group that returned for 5-year assessment.

Missing Data

Of the children who participated in developmental testing (n=138, FT n=57, VPT n= 80), three children (2.1%) were missing SRS total scores, eight children (5.8%) were missing Conners Parent ADHD Index scores, and 15 children (10.9%) were missing CBCL internalizing problems scores. No child was missing more than two out of the three measures, as children were excluded if they did not have data on at least one of the measures of interest. The data were missing due to incomplete forms or failure to return certain questionnaires. Multiple imputation was used to address data missing in the parent-report questionnaires. We performed multiple imputations and

used the aggregate (pooled) data in all statistical tests (1). The means for the pooled data were not significantly different from the means in the original data.

Teacher reports were obtained for 76% of the children with parent-report data (24% missing data). Of the teacher completed questionnaires, 10 (9.5%) were missing TRF Internalizing Scores. All of the children with teacher data had a complete Conners and SRS (n=105). This resulted in a final sample of 95 children with a complete set of teacher questionnaires (FT=32; VPT=63). Missing data were not imputed for teacher reported data since greater than 15% of the children with parent questionnaires were missing teacher questionnaires.

Regression Model Variable Selection

Age at scan, sex, and medical risk were included in the model because they were related to the cingulum bundle (CB) or outcome measures. Specifically, age at scan was related to FA of the right (p=.008) and left (p=.013) anterior CB. Sex (male=0, female=1) was related to MD of the right posterior CB (p=.024) and AD of the right posterior CB (p=.044). Medical risk (a composite measure of perinatal clinical factors including oxygen requirement at 36 weeks postmenstrual age, patent ductus arteriosus treatment with medication and/or ligation, necrotizing enterocolitis, postnatal steroids (dexamethasone), absence of antenatal steroids, confirmed sepsis, upper quartile for total parenteral nutrition, retinopathy of prematurity surgery, intrauterine growth restriction, and significant (>3SD) weight loss from birth to discharge) was related to FA of the left posterior CB (p=.006) and Conners ADHD scores (p=.022). We also examined whether social risk factors (African American race, maternal age <18 years at time of the infant's birth, public insurance status, single parent household, and mother with no high school degree) at age 5 were related to

our diffusion measures or outcome measures, but these variables were not included in the models because they were not related to our variables of interest (p-values ranging from .11-.98).

Principal Component Analyses (PCA)

The PCA was performed on the subset of children with both parent and teacher data on z-scored measures of internalizing, attention, and autism symptoms (n=95, FT n=32, VPT n=63). A PCA with oblinmin rotation with Kaiser normalization was chosen to allow for correlation between factors because we did not expect the factors to be entirely independent (2). The six measures included in the PCA were Child Behavioral Checklist (CBCL) internalizing symptoms, Teacher Response Form (TRF) internalizing symptoms, Conners Parent ADHD Index score, Conners Teacher ADHD Index score, Social Responsiveness Scale (SRS) Parent Total score, and SRS Teacher Total score. The variables loaded onto two factors representing the preterm behavioral phenotype, with parent scores loading strongly onto one factor and teacher scores loading onto the second factor (Table S1). The clustering of parent and teacher scores separately is expected given that differences between parent and teacher reports have been previously reported in the literature (3–5), and suggests that parents and teachers may be capturing context-dependent behaviors.

As the sample size was reduced when both parent and teacher variables were used in the model, separate PCAs for parent and teacher variables were conducted. A PCA was performed on the 5-year parent-report data with 57 FT subjects and 80 VPT subjects. The scores clustered into a single factor, representing 80.4% of the variance with factor loadings of .907, .890, and .893 for the SRS Parent Total z score, Conners Parent ADHD Index z score, and CBCL Internalizing Problems z score, respectively. This factor is referred to as the parent-rated preterm behavioral phenotype score. A second PCA was run with the teacher-reported data (n=95, FT n=32, VPT

n=63). The scores also clustered into a single factor, referred to as the teacher-rated behavioral phenotype score, which explained 79.9% of the variance with factor loadings of .920, .919, and .842 for the Teacher Response Form (TRF) internalizing symptoms, SRS Teacher Total score, and Conners Teacher ADHD Index score, respectively.

Cingulum Bundle and Preterm Behavioral Phenotype Sub-Domains

We examined the relationship between neonatal white matter microstructure of the dorsal anterior CB and each domain of the preterm behavioral phenotype symptoms. Linear regression models adjusted for age at scan, sex, and medical risk were performed in VPT children (n=56 neonatal data + parent outcomes; n=45 neonatal data + teacher outcomes). This analysis was conducted as a follow-up to our findings that the right anterior CB was related to the preterm behavioral phenotype. For the parent-rated preterm behavioral phenotype symptoms, FA in the right anterior CB was related to the Conners ADHD Index z-score, but not related to the SRS Total z-score or CBCL Internalizing Problems z-score after correcting for multiple comparisons (Table S3). For the teacher-rated preterm behavioral phenotype, MD, AD, and RD of the right anterior CB were associated with all three domains: internalizing, ADHD, and autism symptoms (Table S3). FA of the right anterior CB was related to internalizing, but not ADHD or autism symptoms after accounting for multiple comparisons (Table S3).

Specificity: Corpus Callosum and Preterm Behavioral Phenotype

The Corpus Callosum (CC) was used as a control tract because it is a large, medial white matter tract in proximity to the CB and has a similar postnatal developmental trajectory (6). Identical procedures for DTI processing (Methods) were used to extract the FA and MD of the CC

(7). Features of the CC differed between VPT and FT infants. MD was increased (t=-7.747, p<.001) and FA was reduced (t=4.696, p<.001) in VPT compared to FT infants, consistent with prior results in the literature (7,8). Regression models controlling for age at scan, sex, and medical risk were used to assess the association between the CC and the parent-rated and teacher-rated preterm behavioral phenotypes. The FA and MD of the CC were not related to the preterm behavioral phenotype scores using either parent or teacher data after FDR correction for multiple comparisons with beta coefficients ranging from -.032–.289 (p-values =.04–.566). This finding provides some support for the specificity of the relationship between the CB and the preterm behavioral phenotype.

Specificity: Cingulum Bundle and Full-Scale IQ

The children's intellectual ability was assessed at age 5 using the Wechsler Preschool Primary Scales of Intelligence-III, a cognitive assessment with good psychometric properties (9,10). The Full-Scale IQ scores (FSIQ) were used as a proxy for general intellectual ability. FSIQ scores are based upon the subtests: Vocabulary, Word Reasoning, Block Design, Matrix Reasoning, and Picture Concepts. Regression models adjusted for age at scan, sex, and medical risk were used to assess the association between the right CB and FSIQ in VPT children (n=55). Separate regression models were conducted for FA, MD, AD, and RD for the right CB and the right anterior CB. Neither the right CB nor the right anterior CB were significantly associated with FSIQ (p=.230–.651). This finding supports the specificity of the relationship between the CB and the preterm behavioral phenotype.

Supplemental References

1. Rubin, Donald B (1996): Multiple Imputation After 18+ Years. *J Am Stat Assoc* 91: 18.
2. Schmitt TA (2011): Current Methodological Considerations in Exploratory and Confirmatory Factor Analysis. *J Psychoeduc Assess* 29: 304–321.
3. Verhulst FC, Koot HM, Ende J (1994): Differential predictive value of parents' and teachers' reports of children's problem behaviors: A longitudinal study. *J Abnorm Child Psychol* 22: 531–546.
4. O'Meagher S, Norris K, Kemp N, Anderson P (2019): Parent and teacher reporting of executive function and behavioral difficulties in preterm and term children at kindergarten. *Appl Neuropsychol Child* 1–12.
5. Scott MN, Taylor HG, Fristad MA, Klein N, Espy KA, Minich N, Hack M (2012): Behavior Disorders in Extremely Preterm/Extremely Low Birth Weight Children in Kindergarten: *J Dev Behav Pediatr* 33: 202–213.
6. Kinney HC, Ann brody B, Kloman AS, Gilles FH (1988): Sequence of Central Nervous System Myelination in Human Infancy. II. Patterns of Myelination in Autopsied Infants: *J Neuropathol Exp Neurol* 47: 217–234.
7. Dubois J, Dehaene-Lambertz G, Kulikova S, Poupon C, Hüppi PS, Hertz-Pannier L (2014): The early development of brain white matter: A review of imaging studies in fetuses, newborns and infants. *Neuroscience* 276: 48–71.
8. Constable RT, Ment LR, Vohr BR, Kesler SR, Fulbright RK, Lacadie C, *et al.* (2008): Prematurely Born Children Demonstrate White Matter Microstructural Differences at 12 Years of Age, Relative to Term Control Subjects: An Investigation of Group and Gender Effects. *PEDIATRICS* 121: 306–316.
9. Wechsler D (2004): *WPPSI-III Australian Administration and Scoring Manual*. Harcourt Assessment/Psychological Corporation.
10. Lichtenberger EO (2005): General measures of cognition for the preschool child. *Ment Retard Dev Disabil Res Rev* 11: 197–208.

Table S1. Principal Component Analysis with Parents and Teachers

	Factor 1	Factor 2
CBCL Parent Report Internalizing Z-Score	.992	.320
Conners Parent Report ADHD Index Z-Score	.912	.346
SRS Parent Total Z-Score	.926	.274
TRF Teacher Report Internalizing Z-Score	.274	.924
Conners Teacher Report ADHD Index Z-Score	.331	.846
SRS Teacher Total Z-Score	.311	.925
Variance Explained	55.5%	27.2%

Table S2. Neonatal AD and RD Differences in the Cingulum Bundle

	Term (M ± SD) N=55	Preterm (M ± SD) N=69	*p*
Gestational Age at Birth (weeks)	38.9 ± 1.3	26.7 ± 1.8	**<.001**
Gestational Age at Scan (weeks)	38.9 ± 1.2	37.5 ± 1.4	**<.001**
AD of Right CB	1.62 ± .07	1.66 ± .07	**.010**
AD of Right Anterior CB	1.60 ± .08	1.64 ± .08	**.018**
AD of Right Posterior CB	1.63 ± .07	1.69 ± .07	**.001**
AD of Left CB	1.62 ± .07	1.68 ± .07	**.001**
AD of Left Anterior CB	1.60 ± .07	1.75 ± .09	**.015**
AD of Left Posterior CB	1.64 ± .08	1.71 ± .07	**.001**
RD of Right CB	1.23 ± .06	1.27 ± .06	**.026**
RD of Right Anterior CB	1.25 ± .07	1.32 ± .08	**.002**
RD of Right Posterior CB	1.17 ± .06	1.20 ± .06	.075
RD of Left CB	1.21 ± .06	1.27 ± .07	**.002**
RD of Left Anterior CB	1.24 ± .07	1.31 ± .08	**.001**
RD of Left Posterior CB	1.16 ± .06	1.20 ± .05	**.003**
			p
Male/Female	24/31	29/40	.857
African American/Caucasian	40/15	29/34*	**.003**

* 4 Asian & 2 biracial VPT participants; **Bold** indicates p-values that passed FDR correction

Table S3. Regression Models for Specific Domains of the Preterm Behavioral Phenotype

Parent Report						
	CBCL Internalizing		**Conners ADHD**		**SRS Total**	
	β	**p**	**β**	**p**	**β**	**p**
Right Anterior CB FA	.297	.040	.348	**.012**	.153	.307

Teacher Report						
	TRF Internalizing		**Conners ADHD**		**SRS Total**	
	β	**p**	**β**	**p**	**β**	**p**
Right Anterior CB FA	.333	**.021**	.274	.046	.151	.298
Right Anterior CB MD	-.380	**.009**	-.365	**.009**	-.338	**.021**
Right Anterior CB AD	-.413	**.006**	-.356	**.013**	-.444	**.002**
Right Anterior CB RD	-.417	**.004**	-.412	**.003**	-.359	**.014**

Bold indicates p-values that passed FDR correction