A DTI-based tractography study of effects on brain structure associated with prenatal alcohol exposure in newborns

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1) Newborn infant subjects

- 11 prenatally alcohol exposed (PAE):
  - 6 female/5 male; postconception age range 36-44 wk, median 42 wk;
- 9 healthy controls (HC):
  - 3 female/6 male; postconception age range 38-44 wk, median 42 wk.

TABLE 1: Summary of sample characteristics.

	HC (n = 9) Mean/%	SD	PAE (n = 11) Mean/%	SD	p-value
Infant characteristics					
Sex (% female)	33.3		54.5		0.075
Gestational age at birth (wk)	38.7	1.9	38.6	2.0	0.939
Postpartum age at scan (wk)	3.0	1.6	2.6	1.5	0.589
Postconception age at scan (wk)	41.6	2.1	41.2	2.3	0.658
Birthweight (g)	2753.3	478.4	2754.1	413.5	0.997
Maternal characteristics					
Maternal age at delivery (yr)	24.0	4.7	29.8	4.6	0.012 *
Parity	1.2	1.2	2.3	1.5	0.105
Education[1] (yr)	10.6	0.9	9.7	1.0	0.807
Marital status (% married)	33.3		18.2		0.176
Smoking (cig/day)	4.3	2.5	6.5	4.8	0.218
Marijuana (days/wk)	0.0	0.1	0.2	0.5	0.509
Extent of alcohol consumption					
At conception					
oz AA/day[2]	0.02	0.05	2.25	1.89	0.002 **
oz AA/occasion[2]	0.13	0.38	4.70	3.51	0.001 **
frequency (days/wk)	0.02	0.05	0.42	0.30	0.001 ***
Across pregnancy					
oz AA/day[2]	0.01	0.03	1.68	1.52	0.004 **
oz AA/occasion[2]	0.13	0.40	4.66	1.35	0.000 ***
frequency (days/wk)	0.01	0.03	0.33	0.26	0.001 **
Infant global parameters					
Number DWIs[3]	21.7	4.4	22.4	3.6	0.698
Total intracranial volume (cm³)	469.4	60.8	434.6	43.5	0.152
WM volume (cm³)	200.7	23.0	182.9	25.6	0.123
WM fraction	0.43	0.04	0.42	0.03	0.535

HC = healthy control; PAE = prenatal alcohol exposure; SD = standard deviation; AA = absolute alcohol. * p<0.05; ** p<0.01; *** p<0.001.
[1]Education missing for one HC mother.
[2]Measures of oz AA for one PAE mother were Winsorized.
[3]Number of DWIs remaining after deletion of any dropout/motion-corrupted volumes.

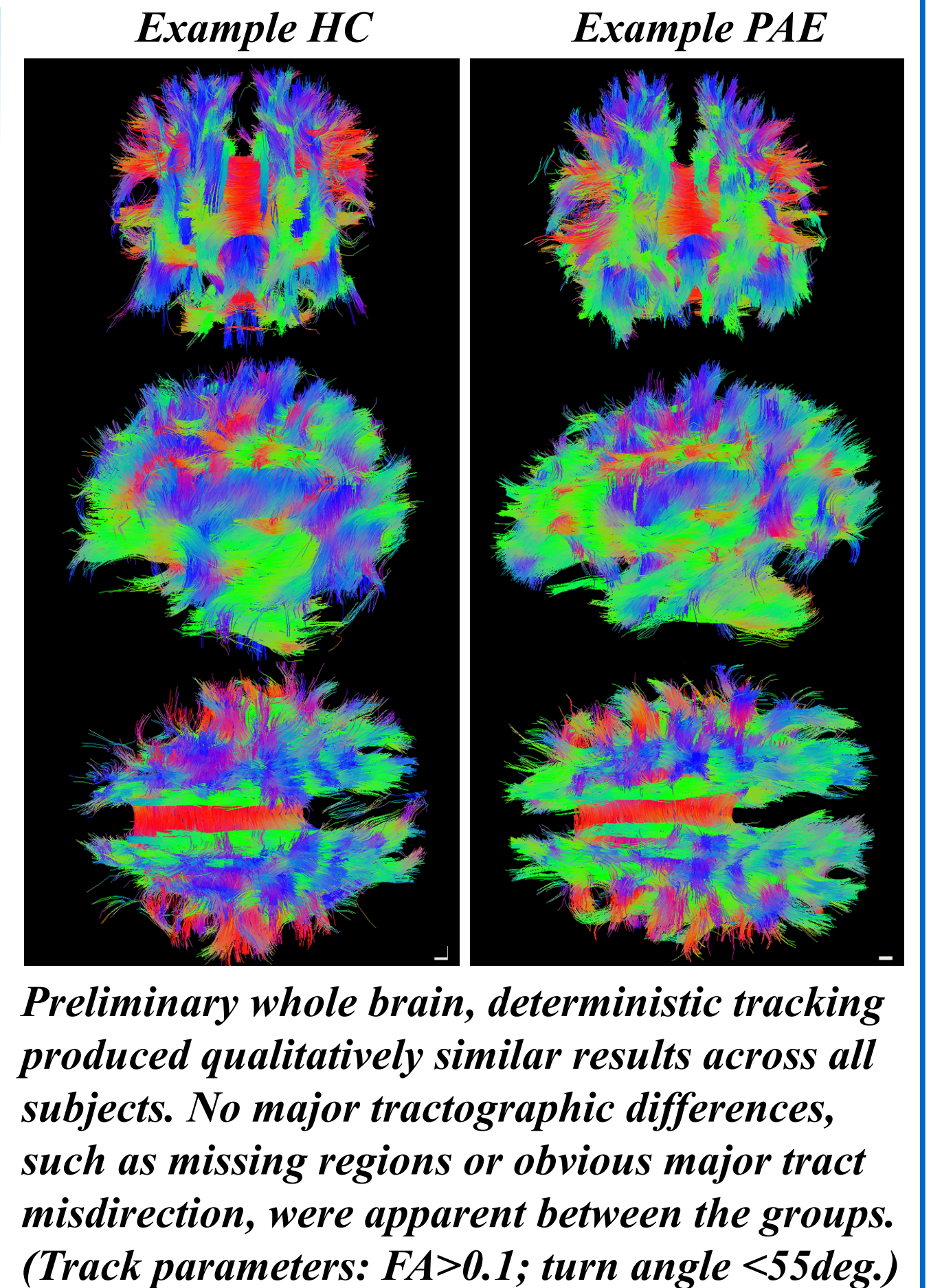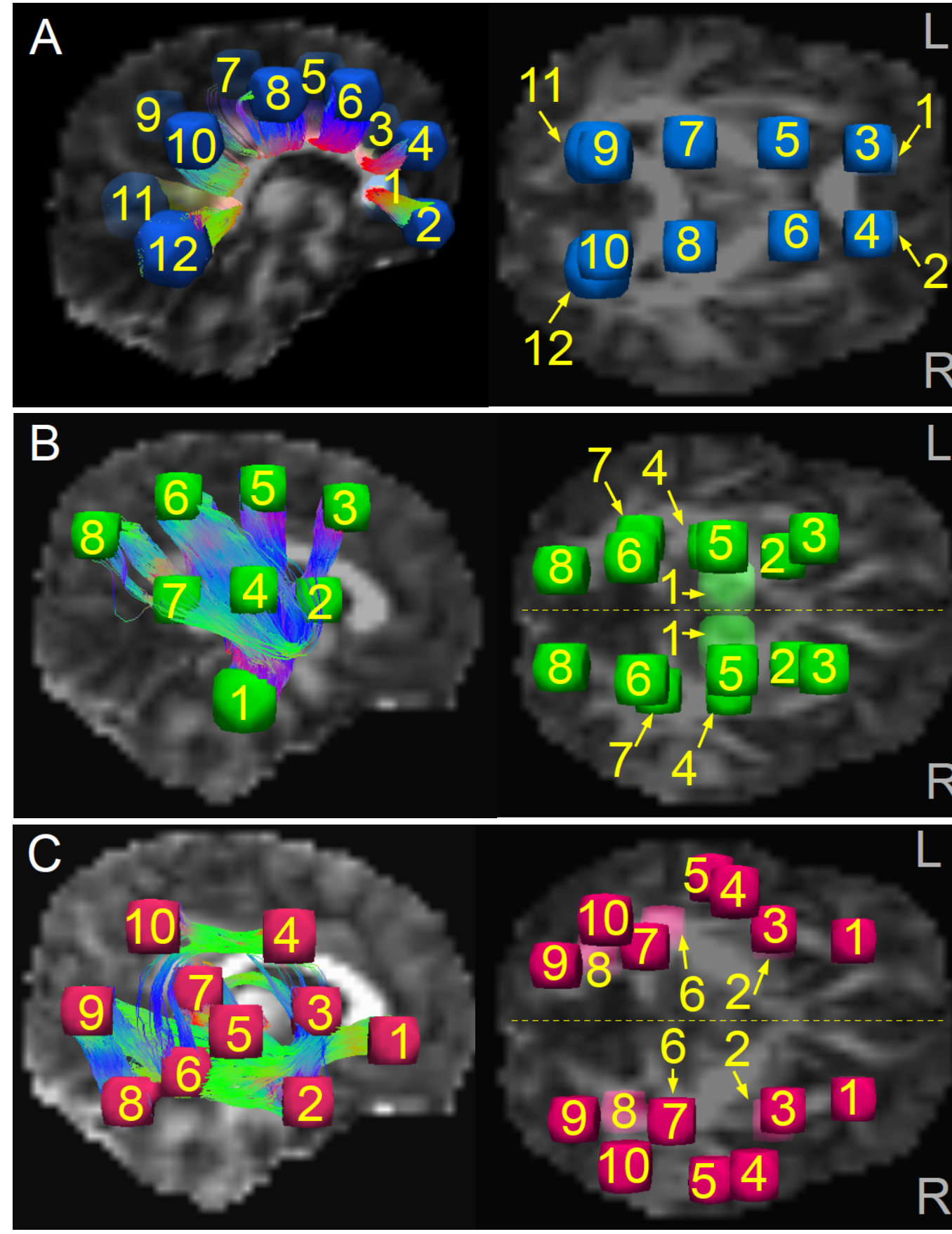## 2) DTI + structural parameters

- Diffusion tensor imaging (DTI)
  - fractional anisotropy (FA); mean, axial and radial diffusivity (MD, AD, RD).
- Structural
  - proton density (PD) and physical T1
  - ROI volume (scaled by whole brain)

Example HC   *Example PAE*
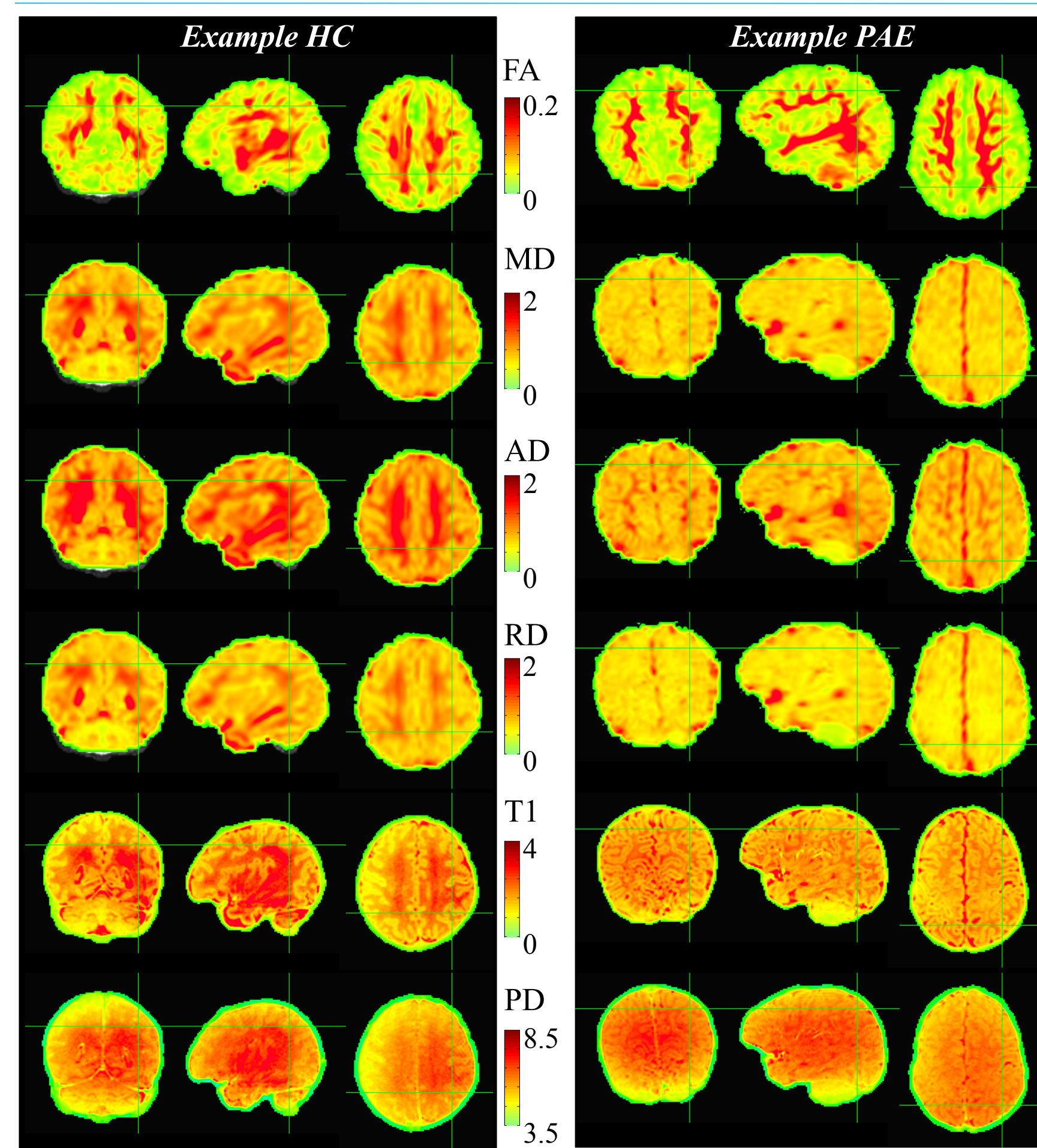
3) Tractography

- AFNI-FATCAT[1,2] used to estimate and delineate similar white matter (WM) ROIs across all subjects.
- Probabilistic tracking in 5 networks (Figure below, left panel):
  A) Transcallosal: corpus callosum and corona radiata (CCCR);
  B) Corticospinal: L and R projection (PROJ) fibers;
  C) Corticocortical: L and R association (ASSOC) fibers.


Locations of target ROIs per WM network   *Example HC*   *Example PAE*

Preliminary whole brain, deterministic tracking produced qualitatively similar results across all subjects. No major tractographic differences, such as missing regions or obvious major tract misdirection, were apparent between the groups. (Track parameters: FA>0.1; turn angle <55deg.)

4) Subject recruitment

Data from three timeline follow-back interviews[3] were combined to provide continuous measures of drinking during pregnancy. Infant exclusionary criteria were major chromosomal anomalies, neural tube defects, multiple births, very low birthweight (< 1500 g), gestational age <32 wks, seizures and major drug usage.

Human subjects approval was obtained from the Wayne State University and University of Cape Town institutional review boards. Informed consent was obtained both at time of recruitment and at time of scan. All women who reported drinking during pregnancy were advised to stop or reduce their intake and were offered referrals for treatment.

5) Data acquisition and processing

No infants were sedated (feeding and swaddling ensured sleep and reduced motion).

Scanning: 3T Siemens Allegra using a custom-built, 170.9 mm (inner diameter) circularly polarized birdcage RF coil.

Two DWI sets were acquired (each: 4 b=0 and 30 b=1000 s mm⁻² images) with opposite phase encoding (AP/PA) using a twice-refocused SE-EPI sequence (2mm isotropic voxels). Processing included motion correction using FSL[4], susceptibility-distortion correction[5,6], and outlier rejection.

Anatomical imaging (1mm isotropic) was performed using a multiecho FLASH sequence[7], and T1 and PD maps reconstructed using Freesurfer's mri_ms_fitparms.

6) WM networks: analysis and statistics

- Tracking connections:
  - many found in all subjects (dark blue, Figure right); → used in analyses;
  - many found in >80% of both groups (light blue);
  - few found only in >80% of single group (orange).

L-PROJ   R-PROJ   L-ASSOC   R-ASSOC
HC / PAE
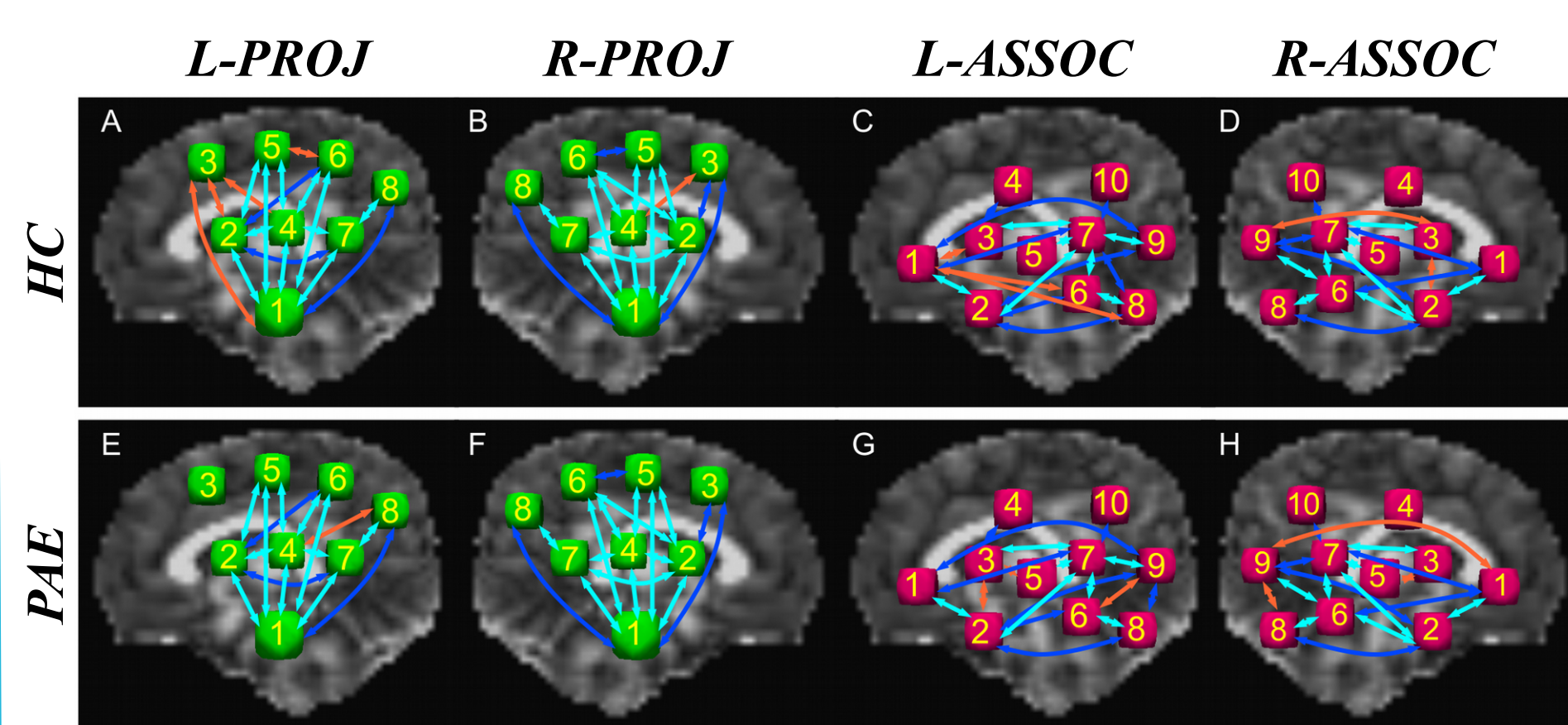
- Network level alcohol-structure relations (Table below):
  - calculated with multivariate GLM (using R package afex[8]).
  - accounted for confounds: infant age and sex; maternal age and cigarettes.
  - no significant relation with FA or ROI volume in any network;
  - AD (axial diffusivity) showed significant relations (decrease with increasing alcohol) in most networks (even after multiple comparison correction).

Network	FA var.	FA β_{med}	FA $F(df_N, df_D)$	FA p	MD var.	MD β_{med}	MD $F(df_N, df_D)$	MD p	AD var.	AD β_{med}	AD $F(df_N, df_D)$	AD p	PD var.	PD β_{med}	PD $F(df_N, df_D)$	PD p
CCCR					alc	-0.70	8.6 (1, 14)	0.011*	alc	-0.72	14.0 (1, 14)	0.002**	cig	0.47	3.5 (1, 14)	0.083
									cig	-0.27	2.5 (6, 9)	0.101				
					mat_age	0.56	5.5 (1, 14)	0.034*	mat_age	0.53	6.3 (1, 14)	0.025*				
L-PROJ	cig	0.12	4.2 (11, 4)	0.091	alc	-0.41	3.9 (10, 140)	0.000***	alc	-0.52	4.1 (10, 140)	0.000***	cig	0.52	4.0 (1, 14)	0.066
									mat_age	0.44	6.5 (1, 14)	0.023*				
					mat_age	0.37	4.4 (1, 14)	0.056								
R-PROJ	age	0.33	8.6 (13, 2)	0.109	alc	-0.41	1.9 (12, 168)	0.035*	alc	-0.45	2.7 (12, 168)	0.002**	cig	0.48	3.4 (1, 14)	0.085
					age	-0.41	5.8 (1, 14)	0.031*	age	-0.39	5.3 (1, 14)	0.038*				
	mat_age	-0.16	9.2 (13, 2)	0.103	sex	-0.20	4.3 (1, 14)	0.056	sex	-0.39	5.1 (1, 14)	0.029*				
L-ASSOC					alc	-0.65	6.0 (7, 8)	0.011*	alc	-0.66	8.1 (1, 14)	0.013*	cig	0.49	3.6 (1, 14)	0.080
									age	-0.16	2.5 (6, 84)	0.030*				
					mat_age	0.44	3.8 (1, 14)	0.071	mat_age	0.43	4.7 (1, 14)	0.048*				
R-ASSOC	alc	0.23	1.8 (7, 98)	0.090	alc	-0.62	10.2 (1, 14)	0.007**	alc	-0.67	14.1 (1, 14)	0.002**	cig	0.52	3.5 (1, 14)	0.082
									age	-0.29	3.9 (1, 14)	0.068				

F-values are shown with final degrees of freedom of numerator (df_N) and denominator (df_D). Median standardized β-values were calculated from the additional multivariate regressions of each network's individual WM-ROIs. Only predictors exhibiting effects at p≤0.10 are shown. * p<0.05; ** p<0.01; *** p<0.001. alc = frequency of maternal drinking; age = infant age since conception; sex: male=0, female = 1; cig = maternal smoking (cig/day); mat_age = maternal age at delivery.

7) Intranetwork statistics for AD relations

- Follow-up, per WM ROI alcohol-structure relations (graphs below):
  - investigate *where* in networks most significant AD-alcohol relations occur.
  - accounted for confounds: infant age and sex; maternal age and cigarettes.
  - β plots show several ROIs with significant AD-alcohol relation (green)
  - strongest relations were observed in medial and inferior WM.

Legend: alc, age, sex, cig, mat_age
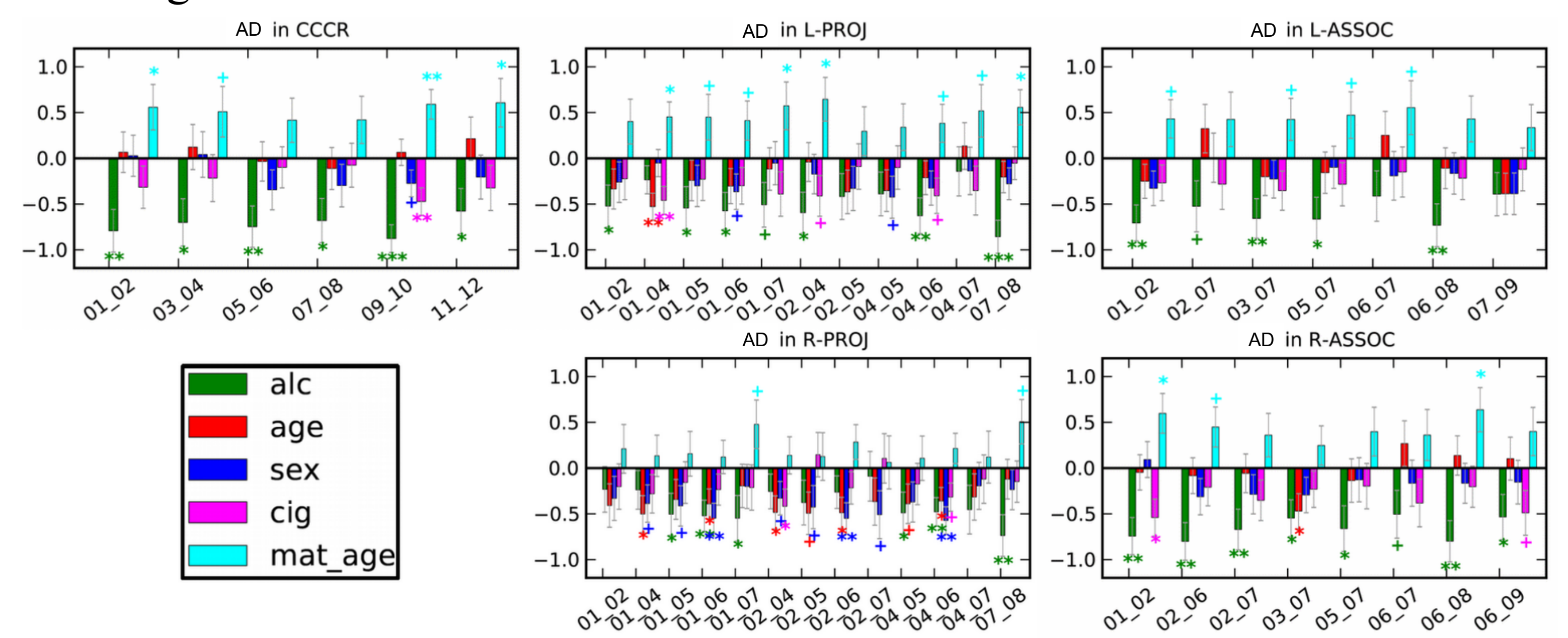
8) Take home conclusions

- PAE in newborns is associated with structural WM changes.
- Changes occur across transcallosal, corticospinal and corticocortical networks
- FA showed no significant alcohol relations
- AD shows significant decrease with increased alcohol exposure in WM networks
- Strongest AD-alcohol relations were seen in medial+inferior WM (likely locations of early maturation).

9) References

[1] Taylor PA, Saad ZS (2013). Brain Connectivity 3(5):523-535. [2] Cox RW (1996). Comput Biomed Res 29:162-173. [3] Jacobson et al., (2008) Alcohol Clin Exp Res. 32:365-372. [4] Smith S, et al., (2004) NeuroImage 23(S1):208-219. [5] Andersson et al., (2003) NeuroImage 20(2):870-888. [6] Rohde et al., (2004) Magn Reson Med 51:103–114. [7] van der Kouwe et al., (2008) Neuroimage 40,559-569. [8] Singmann (2013) http://CRAN.Rproject.org/package=afex.