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Associations between low-moderate prenatal alcohol exposure and brain development in childhood

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ABSTRACT

Background: The effects of low-moderate prenatal alcohol exposure (PAE) on brain development have been infrequently studied.
Aim: To compare cortical and white matter structure between children aged 6 to 8 years with low-moderate PAE in trimester 1 only, low-moderate PAE throughout gestation, or no PAE.
Methods: Women reported quantity and frequency of alcohol consumption before and during pregnancy. Magnetic resonance imaging was undertaken for 143 children aged 6 to 8 years with PAE during trimester 1 only (n = 44), PAE throughout gestation (n = 58), and no PAE (n = 41). T_1-weighted images were processed using FreeSurfer, obtaining brain volume, area, and thickness of 34 cortical regions per hemisphere. Fibre density (FD), fibre cross-section (FC) and fibre density and cross-section (FDC) metrics were computed for diffusion images. Brain measures were compared between PAE groups adjusted for age and sex, then additionally for intracranial volume.
Results: After adjustments, the right caudal anterior cingulate cortex volume (p_{FDR} = 0.045) and area (p_{FDR} = 0.008), and right cingulum tract cross-sectional area ($p_{FWE} < 0.05$) were smaller in children exposed to alcohol throughout gestation compared with no PAE.
Conclusion: This study reports a relationship between low-moderate PAE throughout gestation and cingulate cortex and cingulum tract alterations, suggesting a teratogenic vulnerability. Further investigation is warranted.

1. Introduction

Prenatal alcohol exposure (PAE) is neurotoxic to the fetus, disrupting neuronal proliferation, migration, and glial functioning at a sensitive stage of brain development(Goodlett and Horn, 2001; Lebel et al., 2011). This is a public health concern because many women drink alcohol around conception and/or during pregnancy(Muggli et al., 2016b; Popova et al., 2017). At high or chronic levels, PAE may cause Fetal Alcohol Spectrum Disorder (FASD), characterized by neurodevelopmental impairments and sometimes associated with structural brain abnormalities, facial dysmorphology, and neurological problems (Goodlett and Horn, 2001; Riley et al., 2011).

Abbreviations: AQUA, asking questions about alcohol in pregnancy; CI, confidence interval; DTI, diffusion tensor imaging; FASD, fetal alcohol spectrum disorder; FBA, fixel-based analyses; FC, fibre cross section; FD, fibre density; FDC, fibre density and cross section; FOD, fibre orientation distribution; FOV, field of view; FWE, family wise error; gAA, grams absolute alcohol; GLM, general linear model; ICV, intracranial volume; IQ, intelligence quotient; IQR, interquartile range; M, mean; MRI, magnetic resonance imaging; PAE, prenatal alcohol exposure; QUAD, quality assessment for diffusion magnetic resonance imaging; SD, standard deviation; SQUAD, study-wise quality assessment for diffusion magnetic resonance imaging; TE, echo time; TR, repetition time; WM, white matter.

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Magnetic resonance imaging (MRI) can be used to understand the neurological basis of brain abnormalities associated with PAE. However, radiological findings on routine clinical MRI vary considerably in FASD patients, are dependent on timing, dose and duration of PAE, and do not reveal consistent brain abnormalities that can be used diagnostically (Treit et al., 2020). Alternatively, advanced quantitative MRI research may uncover a pattern of brain structural changes associated with PAE. Indeed, reviews of structural MRI analyses in children following PAE show that brain volume reductions occur most commonly in the corpus callosum, frontal, temporal and parietal lobes, basal ganglia (particularly the caudate) and hippocampus(Donald et al., 2015a; Lebel et al., 2011; Nakhid et al., 2022). There is also evidence for amygdala(McLachlan et al., 2020), cerebellar(Boronat et al., 2017; Meintjes et al., 2014), anterior cingulate and superior frontal gyrus involvement(Andre et al., 2020).

Cortical morphometry has also helped to identify abnormal neuroanatomical features in populations with PAE, with reports of global (Zhou et al., 2018) and regional cortical thinning, especially in medial frontal and parietal regions, in children with FASD(Treit et al., 2014). This appears to be dose-dependent(Robertson et al., 2016) and has even been reported in young adults with low-moderate PAE(Eckstrand et al., 2012). Furthermore, reduced cortical folding has been described in children with FASD(De Guio et al., 2014). Similarly, in children with heavy PAE (>13 drinks/week or > 4 drinks/occasion at least once per week), decreased cortical gyrification has been reported throughout the cortex(Hendrickson et al., 2017), as has reduced surface area of the cortex(Gross et al., 2018), and atypical longitudinal developmental trajectories for gyrification, cortical thickness and volume(Hendrickson et al., 2018).

Prior studies also commonly report that high levels of PAE are associated with altered white matter (WM) microstructure, particularly in the corpus callosum and cerebellar tracts(Donald et al., 2015a; Ghazi Sherbaf et al., 2019; Lebel et al., 2011; Wozniak and Muetzel, 2011). Lower fractional anisotropy on diffusion tensor imaging has also been reported in children with high levels of PAE in anterior-posterior fiber bundles(Wozniak and Muetzel, 2011), corticospinal tracts, cingulum, uncinate fasciculus(Andre et al., 2020), superior longitudinal fasciculus (Paolozza et al., 2017), and inferior longitudinal fasciculus(Fan et al., 2016). Associations between PAE and reduced white matter microstructure have been observed in the newborn period, in locations consistent with older children diagnosed with FASD(Donald et al., 2015b), and interestingly in medial and inferior white matter regions where myelination begins(Taylor et al., 2015).

The diffusion tensor imaging (DTI) model has been used to examine WM microstructure in relation to PAE(Wozniak and Muetzel, 2011), however this model has limitations because the fractional anisotropy measure cannot specifically distinguish myelination, axon density, fiber geometry (e.g., fiber crossings, dispersion) and membrane permeability (Dhollander et al., 2021a; Jones et al., 2013). Furthermore, DTI is unable to reconcile multiple fibre orientations within a single voxel, which are found in the vast majority (≈90 %) of white matter voxels(Jeurissen et al., 2013), including tracts implicated in PAE. Advanced MRI techniques may provide further insights into the effects of PAE on the developing brain. Fixel-based analysis (FBA)(Dhollander et al., 2021a; Raffelt et al., 2017) provides more specific and biologically interpretable measures than DTI by quantifying the microstructural and macrostructural properties of individual WM fiber populations within a voxel, referred to as 'fixels'. Using this technique, measures of fiber density (FD) estimate microscopic density of a particular fiber population within a voxel, fiber cross-section (FC) measures macrostructural changes in the cross-sectional area perpendicular to a fiber bundle, and the combined fiber density and cross-section measure (FDC) is related to the capacity to transfer information across the white matter. As the neurobiological bases for white matter developmental alterations following PAE are still largely unknown, FBA has the potential to identify whether specific white matter fibers experience microstructural axonal loss or macroscopic fiber reduction following PAE, as has been elucidated for other neurodevelopmental disorders, including autism spectrum disorder(Kirkovski et al., 2023), attention deficit hyperactivity disorder (Hyde et al., 2021), and preterm birth(Kelly et al., 2020).

Most MRI research to date has focused on the effects of heavy PAE and FASD on the brain. Few studies have investigated if *low to moderate* levels of alcohol intake during pregnancy are related to offspring's structural brain development and current research is conflicting(Romer et al., 2020). Of those, some are in line with the FASD research, reporting reduced corpus callosum size (Chandran et al., 2021) and lower fractional anisotropy in several white matter regions(Long and Lebel, 2022) following low level PAE compared with unexposed controls. However, another study reported *larger* cerebral and regional cortical volumes and larger regional surface area throughout the temporal, occipital, and parietal lobes for those with low-moderate PAE (Lees et al., 2020). Further study of the effects of low-moderate PAE on the brain is warranted.

The current study will address knowledge gaps by using advanced MRI brain imaging in children with low-moderate PAE at age 6 to 8 years who are part of the Asking Questions about Alcohol in Pregnancy (AQUA) study. In the AQUA study over half (59 %) of mothers consumed some alcohol during pregnancy(Muggli et al., 2016a), and at 12 months of age, low-moderate PAE was associated with subtle midface changes on 3D craniofacial shape analysis in their children(Muggli et al., 2017). However, at two years of age, developmental assessments showed no evidence of poorer cognitive, language or motor outcomes in children with PAE(Halliday et al., 2017). Advanced structural MRI at 6- to 8-years of age will provide additional information about the effects of low-moderate PAE on brain structure, and inform the debate on the safety of low-moderate alcohol consumption in pregnancy and potential preventative strategies(Muggli et al., 2022a).

The aim of this study was to compare cortical and fixel-based white matter development between three groups of children aged 6 to 8 years; those who had (1) low-moderate PAE in trimester 1 only, (2) low-moderate PAE throughout gestation, or (3) no PAE. We hypothesised a dose and timing dependent effect of PAE on brain development (Guerri, 1998), where those with low-moderate PAE throughout gestation and those with PAE in trimester 1 only would have reduced cortical volume, folding and surface area, and poorer white matter microstructural development compared with controls with no PAE. We also hypothesised that children exposed throughout gestation would have more disrupted structural brain development compared with those exposed in trimester 1 only. We hypothesised that corpus callosum, frontal, temporal and parietal, basal ganglia and hippocampal brain regions and their connections would be altered in the PAE compared with no PAE groups, based on the FASD literature described above.

2. Materials and methods

2.1. Subjects

The AQUA prospective longitudinal cohort study recruited 1570 women with singleton pregnancies from seven public hospital sites in Melbourne Australia between July 2011 and July 2012. The study was designed to assess the effects of low-moderate PAE on long-term child development. Women completed questionnaires detailing the quantity, frequency and type of alcoholic beverage consumption three months before pregnancy and in each trimester of pregnancy. A maximum weekly intake, the amount of absolute alcohol in grams (gAA) consumed, was calculated as previously described(Muggli et al., 2016a). Levels of consumption/exposure were defined as low (≤20 g absolute alcohol (AA)/occasion and ≤ 70gAA/week); moderate (21-49gAA/occasion and ≤ 70gAA/week); high (<50gAA/occasion and > 70gAA/week); and binge (≥50gAA/occasion). The present study compared three groups: low-moderate PAE in trimester 1 only (PAE T1); low-moderate PAE throughout gestation (PAE T1-T3); unexposed controls

(no PAE)(Muggli et al., 2022a). At age 6 to 8 years, a PAE-representative subset of children was sequentially invited to have a brain MRI scan, with a target number of 50 in each of the three exposure groups for adequate power to detect group differences(Muggli et al., 2022a). Extensive demographic and socio-environmental factors that may confound or modify the relationship between PAE and child outcomes were also collected at several timepoints. The study was approved by the Human Research and Ethics Committee of the Royal Children's Hospital Melbourne (approval #38025). Parents gave written informed consent for their child to participate.

2.2. Magnetic resonance imaging

Images were acquired using a 3 Tesla Siemens Magnetom Prisma scanner (Erlange, Germany) with a 32 channel head coil, and included a T_1-weighted multi-echo MP-RAGE sequence with echo planar image-navigated prospective motion compensation (repetition time (TR) 2550 ms, echo time (TE)1 2.14, TE2 3.94, TE3 5.77, TE4 7.5 ms, Flip angle 8°, field of view (FOV) = 256 × 256 mm, matrix = 288 × 288, 0.9 mm^3 isotropic voxels), and a diffusion-weighted sequence (TR 3500 ms, TE = 67 ms, FOV = 250 × 250 mm, matrix = 124 × 124, 2.0 mm^3 isotropic voxels, b = 2800 s/mm^2 with 60 gradient directions and 10 b = 0 images, and including a set of reverse phase encoded sequences to correct for *B*0 field inhomogeneity).

T_1-weighted images were processed using FreeSurfer version 7.1.1 (Fischl, 2012), obtaining total and subcortical brain volumes(Fischl et al., 2002), and area, thickness and volume of 34 cortical regions per hemisphere defined using the Desikan-Killiany atlas(Desikan et al., 2006). Brain parcellations and cortical surfaces were inspected and manually corrected as required, according to FreeSurfer guidelines.

Diffusion MRI data was processed using MRtrix3(Tournier et al., 2019), MRtrix3Tissue (https://3Tissue.github.io, a fork of MRtrix3), and the FSL packages(Jenkinson et al., 2012). Typical fixel-based analysis pipeline steps were performed(Dhollander et al., 2021a), including Gibbs-ringing correction(Kellner et al., 2016), eddy current-induced distortions, motion and susceptibility-induced distortion correction (Andersson et al., 2018; Andersson et al., 2017; Andersson et al., 2016; Andersson and Sotiropoulos, 2016), brain extraction(Jenkinson et al., 2005), estimation and averaging of 3-tissue response functions(Dhollander et al., 2019), upsampling to 1.5 mm isotropic voxels, 3-tissue constrained spherical deconvolution(Dhollander and Connelly, 2016), intensity normalisation (global and bias fields)(Dhollander et al., 2021b), and construction of and registration to a study-specific WM fibre orientation distribution (FOD) template(Raffelt et al., 2011). Image quality control was performed by visual inspection and automatically using the FSL Quality Assessment for dMRI (QUAD) and Study-wise Quality Assessment for dMRI (SQUAD) tools(Bastiani et al., 2019). Participants with severe movement artefact in their diffusion images were excluded. Fixel-wise metrics (FD, FC in log form, FDC) were calculated as previously described(Raffelt et al., 2017).

2.3. Statistical analyses

Total and regional brain volumes, cortical area and thickness metrics were compared between PAE groups using general linear models (GLM) adjusted for age and sex to account for sexual dimorphism of brain structural effects of PAE(Treit et al., 2017), and secondarily adjusted for intracranial volume (ICV) to verify regional vulnerability over and above known differences in ICV based on PAE(Donald et al., 2015a; Lebel et al., 2011). Volume, area and thickness variables were standardised relative to the mean and SD of the brain region. *P*-values were corrected for the false discovery rate to account for the multiple brain regions compared within each model. Stata software *v14.2* was used for all analyses involving volumes and cortical metrics(StataCorp, 2015). At each fixel, FD, FC and FDC were compared between PAE groups using a GLM, adjusted for age and sex, and additionally ICV. Connectivity-based smoothing and statistical inference were performed using connectivity-based fixel enhancement(Raffelt et al., 2015). Non-parametric permutation testing (5000 permutations) was used to generate a family-wise error rate (FWE)-corrected *p*-value for every individual fixel. As additional sensitivity analyses, all statistical analyses were also adjusted for prenatal binge-level alcohol exposure before pregnancy recognition, child ethnicity, family structure, maternal education, and financial situation as potential confounders. Partial eta-squared (η^2) values are presented as measures of effect size for brain volume and morphology metrics, calculated as $\eta^2 = SS_{effect} / (SS_{effect} + SSerror)$, where SS_{effect} is the sum of squares for the PAE variable, and SS_{error} is the sum of squares error in the model. $\eta^2 = 0.01$ indicates a small effect, $\eta^2 = 0.06$ indicates a medium effect, and $\eta^2 = 0.14$ indicates a large effect. Percentage effect sizes were calculated using the standard fixel-based analysis pipeline for WM micro- and macrostructure metrics(Dhollander et al., 2021a). All statistical analyses used $p < 0.05$ as the threshold for statistical significance.

3. Results

3.1. Subjects

A total of 146 scans were completed; no PAE $n = 42$, PAE T1 $n = 51$ and PAE T1-T3 $n = 53$. Of these children, 143 had sufficient quality MRI scans to determine brain volume and obtain cortical morphology data (no PAE $n = 41$, PAE T1 $n = 50$, and PAE T1-T3 $n = 52$) and 129 were included in fixel-based analyses (no PAE $n = 37$, PAE T1 $n = 47$, and PAE T1-T3 $n = 45$). Participant characteristics are reported in Table 1. In line with the wider cohort(Muggli et al., 2016a), children in the PAE T1-3 group were more likely to be of white/Caucasian ethnicity, to be living in a shared custody family setting and had a higher proportion of tertiary educated mothers. There was a higher proportion of girls in the PAE T1 group. Fewer mothers smoked in pregnancy in the PAE T1 group, and the number of mothers currently smoking at the 6–8 year follow-up was low across all PAE groups. Other sociodemographic characteristics, including whether the child had any special health care needs, between the three PAE groups were reasonably equally distributed.

Exposure levels in the PAE T1 group reduced to almost zero from a mean (SD) of 26 (73) gAA/weeks following pregnancy recognition, which was usually at around 5 weeks' gestation(Muggli et al., 2016a). While exposure occurred throughout pregnancy in the PAE T1-3 group, levels also reduced dramatically once the mother found out that she was pregnant.

3.2. Brain volumes

ICV was larger in the PAE T1-T3 group compared with the no PAE group ($\eta^2 = 0.11$, $p_{FDR} = 0.017$) (Fig. 1). Before adjusting for ICV, total brain tissue ($\eta^2 = 0.064$, $p_{FDR} = 0.046$), left ($\eta^2 = 0.068$, $p_{FDR} = 0.046$) and right ($\eta^2 = 0.074$, $p_{FDR} = 0.046$) cortical grey matter, left ($\eta^2 = 0.11$, $p_{FDR} = 0.017$) and right ($\eta^2 = 0.089$, $p_{FDR} = 0.036$) caudate, left accumbens ($\eta^2 = 0.081$, $p_{FDR} = 0.043$), left amygdala ($\eta^2 = 0.065$, $p_{FDR} = 0.046$) and brainstem ($\eta^2 = 0.065$, $p_{FDR} = 0.046$) volumes were also larger in the PAE T1-T3 than the no PAE group (Fig. 1). There was little evidence for volumetric differences between the PAE T1 and no PAE group, or the PAE T1 and PAE T1-3 group (all $p_{FDR} \geq 0.05$) (Fig. 1). After adjusting for ICV, there was little evidence for differences in volumes between PAE groups (all $p_{FDR} \geq 0.05$) (Fig. 1). Results were similar after additionally adjusting for binge-level exposure and socio-economic characteristics (Fig. A.1).

3.3. Cortical morphology

There was little evidence for differences in volume, area or thickness between PAE groups for any cortical regions before adjusting for ICV (all

Table 1
Participant Characteristics.

		Volumes & morphometry cohort (*n*=143)						WM[1] micro- & macrostructure cohort (*n*=129)					
		No PAE[2]		PAE T1[3]		PAE T1-3[4]		No PAE		PAE T1		PAE T1-3	
		n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Number of MRIs[5]		41	28.7	50	35.0	52	36.7	37	28.7	47	36.4	45	34.9
Child sex	*Female*	17	41.5	30	60.0	22	42.3	18	48.6	29	61.7	19	42.2
Child ethnicity	*White/Caucasian*	29	70.7	39	79.6	46	88.5	26	70.3	36	78.3	41	91.1
Child has any special health care needs	CSHCN summary score[6]	11	26.8	10	20.0	10	19.2	11	29.7	11	23.4	11	24.4
PAE binge episode	*Yes*			10	20.0	13	25.0			9	19.1	13	28.9
Maternal smoking during pregnancy	*Yes*	6	14.6	2	4.0	7	13.5	6	16.2	3	6.4	7	15.6
Maternal current smoking	*Yes*	2	4.9	1	2.0	2	3.9	2	5.4	1	2.1	2	4.4
Maternal education	*High school*	2	4.9	6	12.0	1	2.0	2	5.4	6	12.8	1	2.2
	Trade/diploma	17	41.5	10	20.0	9	17.7	14	37.8	8	17.0	9	20.0
	Tertiary	22	53.7	34	68.0	41	80.4	21	56.8	33	70.2	35	77.8
Family structure	*Nuclear, dual caregiver*	32	80.0	47	94.0	41	80.4	30	81.1	43	93.5	34	75.6
	Separated, shared custody	4	10.0	2	4.0	9	17.7	3	8.1	2	4.4	10	22.2
	Sole parent	4	10.0	1	2.0	1	2.0	4	10.8	1	2.2	1	2.2
Family financial situation	*Doing alright*	22	53.7	25	50.0	18	35.3	20	54.1	24	51.1	15	33.3
	Living comfortably	12	29.3	21	42.0	24	47.1	11	29.8	19	40.4	22	48.9
	Finding it difficult	7	17.1	4	8.0	9	17.6	6	16.2	4	8.5	8	17.8
		M[7]	(SD)[8]	M	(SD)	M	(SD)	M	(SD)	M	(SD)	M	(SD)
Child age at MRI	*Years*	7.2	0.4	7.2	0.3	7.2	0.3	7.2	0.2	7.2	0.2	7.2	0.2
Child IQ[9]	*Composite score*	104.6	12.6	106.3	11.2	108.1	12.6	104.1	12.8	106.1	11.4	107.7	12.6
Maternal age at birth	*Years*	32.6	5.3	32.7	4.6	33.8	4.5	32.7	5.0	32.3	4.7	34.0	4.5
PAE T1, pre-pregnancy recognition	*absolute alcohol, grams/ week*			25.9	73.2	38.3	49.0			25.5	72.5	37.6	48.8
PAE T1, post-pregnancy recognition	*absolute alcohol, grams/ week*			0.1	0.5	6.6	19.4			0.1	0.4	6.4	19.3
PAE T2	*absolute alcohol, grams/ week*					9.1	19.9					8.9	18.8
PAE T3	*absolute alcohol, grams/ week*					6.3	10.8					6.2	10.7
Maternal current alcohol use	*AUDIT-C[10] summary score*	2.0	1.8	2.9	1.9	3.5	1.7	2.0	1.8	2.6	1.7	3.4	1.7
General family functioning	McMaster[11] *summary score*	1.6	0.5	1.5	0.4	1.6	0.5	1.6	0.5	1.4	0.4	1.7	0.5

[1]WM: white matter.
[2]PAE: prenatal alcohol exposure.
[3] T1: trimester one.
[4] T1-3: trimesters one to three.
[5] MRI: magnetic resonance imaging.
[6] CSHCN: Child Special Health Care Needs Screener (Bethell, C.D., et al. 2002).
[7] M: Mean.
[8] SD: Standard deviation
[9] IQ: intelligence quotient, measured using the Wechsler Intelligence Scale for Children (WISC-V Australian & New Zealand Standardised Edition)(Wechsler, 2014).
[10] AUDIT-C: Derived Alcohol Use Disorders Identification Test (Dawson, D.A., et al 2005).
[11] McMaster: McMaster Family Assessment Device; General family functioning sub-scale (Epstein et al 1983).

$p_{FDR} \geq 0.05$; data not shown). After adjusting for ICV, the volume ($\eta^2 = 0.11$, $p_{FDR} = 0.045$) and area ($\eta^2 = 0.15$, $p_{FDR} = 0.008$) of the right caudal anterior cingulate cortex was lower in the PAE T1-T3 group compared with the no PAE group, with little evidence for differences between PAE groups for any other cortical metrics (Fig. 2). Results were similar after additionally adjusting for binge-level exposure and socio-economic characteristics, except differences between the PAE T1-T3 group compared with the no PAE group for right caudal anterior cingulate after adjusting for ICV diminished for both volume ($\eta^2 = 0.090$, $p_{FDR} = 0.3$) and area ($\eta^2 = 0.12$, $p_{FDR} = 0.06$) (Fig. A.2).

3.4. WM microstructure and macrostructure

On whole-brain fixel-wise analysis, there was little evidence for differences in FD or FDC between PAE groups (all $p_{FWE} \geq 0.05$). The PAE T1 group exhibited higher FC than the no PAE group in small clusters of fixels located in the cerebellar WM and brainstem before adjusting for ICV (all $p_{FWE} < 0.05$; Fig. 3, top) but not after adjusting for ICV. The PAE T1-T3 group had higher FC than the no PAE group in the anterior limb of the internal capsule and genu of the corpus callosum before (all $p_{FWE} < 0.05$; Fig. 3, middle), but not after adjusting for ICV. After adjusting for ICV, the PAE T1-T3 group exhibited lower FC in a small region located in right cingulum bundle compared with the no PAE group ($p_{FWE} < 0.05$; Fig. 3, bottom). When additionally adjusting for binge-level exposure and socio-economic characteristics, the PAE T1-T3 group still had lower FC in the right cingulum compared with the no PAE group (Fig. A.3).

4. Discussion

4.1. Summary

Overall, when considering the effects of low-moderate PAE on the brain at 6- to 8-years of age, there were few differences compared with unexposed controls, particularly for the PAE T1 only group. Differences in brain regions and tracts that were identified between the PAE T1-3 and no PAE group largely attenuated after accounting for the differences in overall brain size between the PAE groups. ICV, the cortex and some subcortical grey matter structures were larger in the PAE T1-T3 than no PAE group. However, after accounting for ICV, the volume

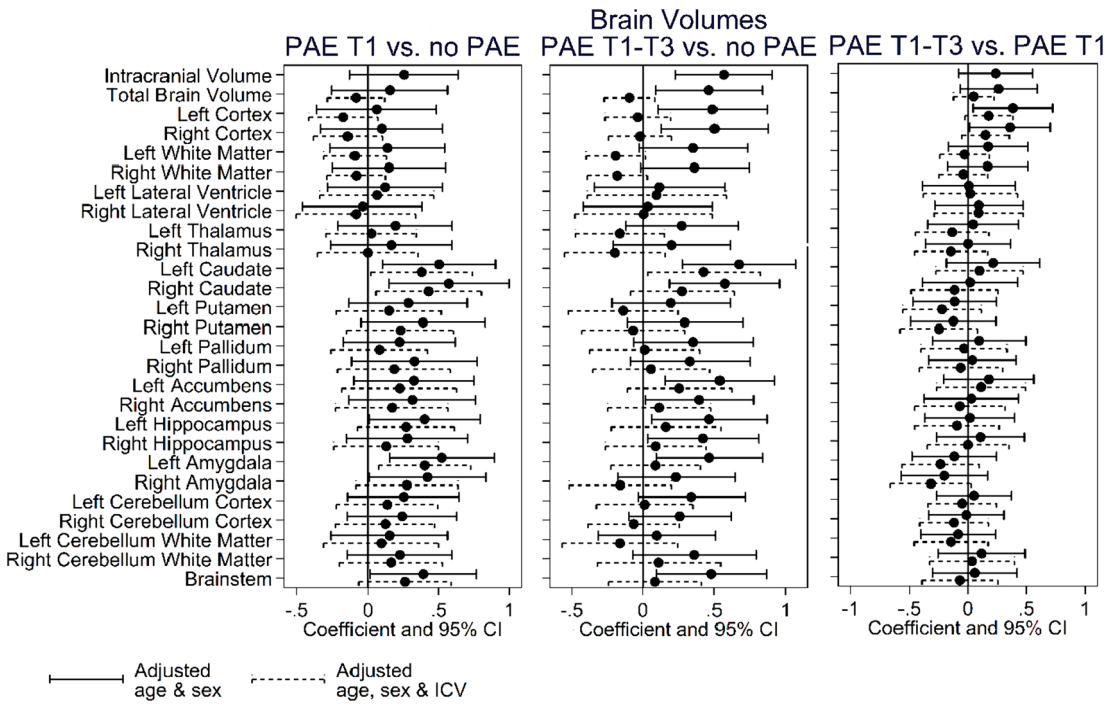

Fig. 1. Points indicate standardised beta coefficients for mean **total brain and subcortical volume** differences between prenatal alcohol exposure (PAE) groups, adjusted for age and sex (solid line) or additionally adjusted for intracranial volume (ICV) (dashed line). Error bars are 95% confidence intervals (CI). NOTE points left of zero line denote *lower* volumes in first group than comparison group; points right of zero line denote *higher* volumes in first group than comparison group.

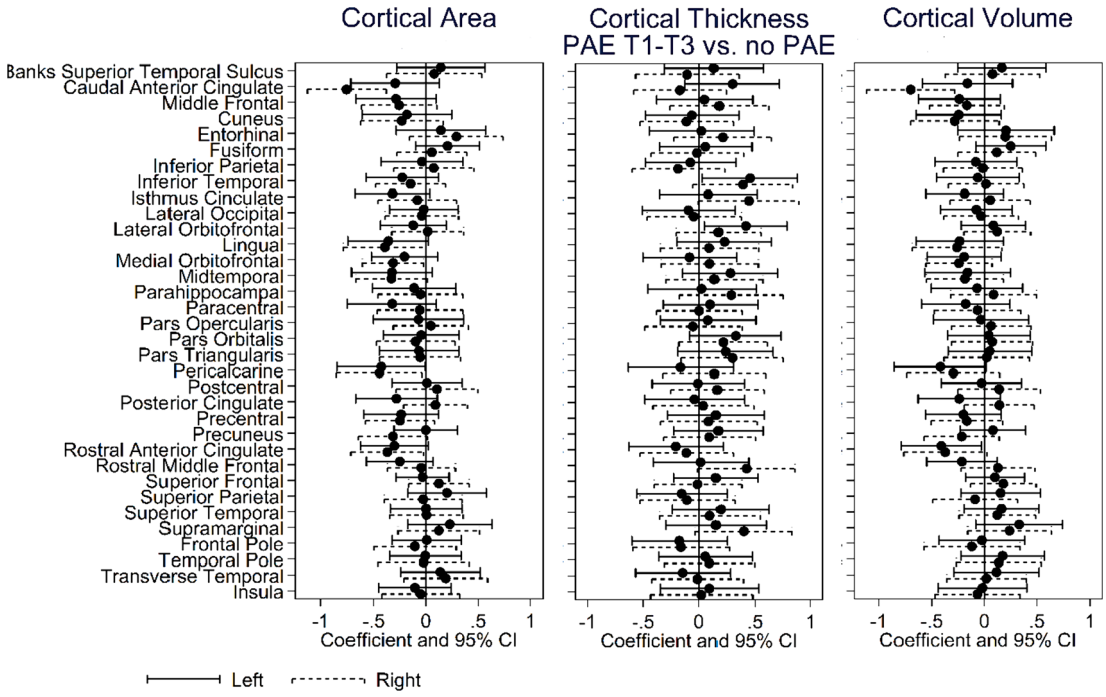

Fig. 2. Points indicate standardised beta coefficients for mean **regional cortical area, thickness and volume** differences between children with low-moderate prenatal alcohol exposure throughout trimesters 1 to 3 (PAE T1-T3) and children with no prenatal alcohol exposure (no PAE), adjusted for age, sex and intracranial volume for the left (solid line) and right hemispheres (dashed line). Error bars are 95% confidence intervals (CI). NOTE points left of zero line denote *lower* volumes in the PAE T1-T3 group than no PAE group; points right of zero line denote *higher* volumes in PAE T1-T3 group than no PAE group.

and surface area of the right caudal anterior cingulate cortex and the cross-sectional area of right cingulum bundle were smaller in the children exposed to low-moderate alcohol throughout gestation (PAE T1-T3) than in the no PAE group suggesting potential regional teratogenic vulnerability. Nevertheless, differences in socio-economic factors between the PAE groups may have contributed to some of these findings.

4.2. Brain volumes

Most previous research has reported smaller volumes of most brain

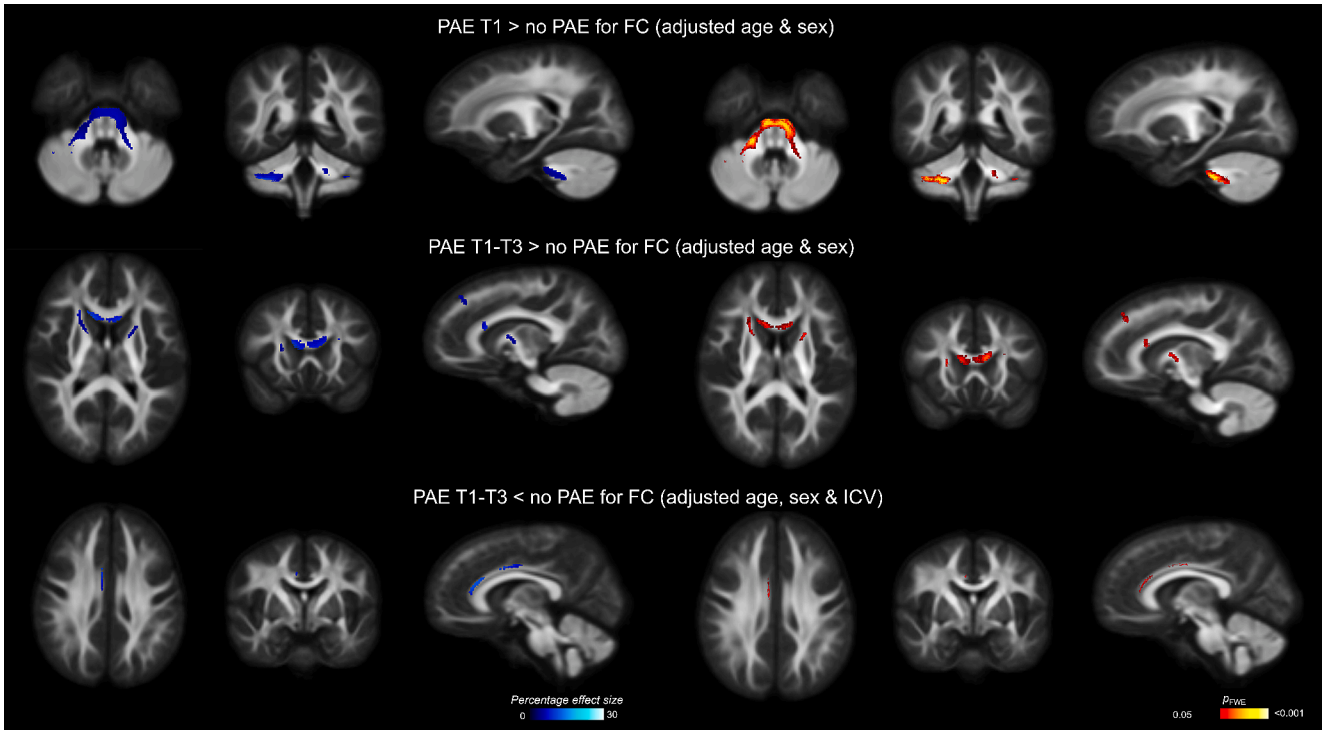

Fig. 3. Fixel-based analysis results for fibre cross-section (FC). Top row: Cerebellar white matter and brainstem fibres passing through fixels with higher FC in the prenatal alcohol exposure (PAE) trimester 1 only (T1) group than the no PAE group before adjusting for intracranial volume (ICV). Middle row: Anterior limb of the internal capsule and genu of the corpus callosum fibres passing though fixels with higher FC in the PAE throughout trimesters 1 to 3 (T1-T3) group than the no PAE group before adjusting for ICV. Bottom row: Fibres from the right cingulum passing through fixels with lower FC in the PAE trimesters 1 to 3 (T1-T3) group than the PAE T1 group only after adjusting for intracranial volume.

structures in children with heavy PAE compared with controls(Zhou et al., 2018), including total brain volumes(Donald et al., 2015a; Lebel et al., 2011). Others have found larger ICV and regional volumes after *low-moderate* PAE(Lees et al., 2020). Similarly, we found increased ICV, total brain volume, brainstem, bilateral cortex and caudate, and left accumbens and amygdala volumes in children with low-moderate PAE in T1-T3 relative to controls. This may indicate brain 'sparing' or a compensatory response to counter the effects of alcohol in other brain regions, or in the case of a larger cortex, possible incomplete cortical pruning(Nunez et al., 2011). Either way, more volume may not necessarily be better. There was little evidence for the regional volumetric increases we found after adjusting for ICV, meaning these regions were not disproportionately affected by PAE over and above its effect on total brain size. Although smaller brain volumes are generally associated with poorer neurodevelopmental outcomes, our research found little evidence for differences in neurodevelopmental outcomes between PAE groups in this cohort at age 2 years(Halliday et al., 2017), or in IQ at age 6 to 8 years (Table 1). Thus, we lack evidence for differences between PAE groups in either brain volume or function in this cohort.

4.3. Cortical morphology

After adjusting for ICV, the volume and surface area of the right caudal anterior cingulate cortex was lower in the PAE T1-T3 group than the unexposed control group. Thus, after accounting for a child's overall brain size, the size of this cortical region appeared impacted by low-moderate PAE throughout gestation. A smaller surface area may result from interruption to migration of radial cells(Bosco and Diaz, 2012), may correspond to a reduction in neurons(Rakic, 2004), or may reflect decreased cortical complexity and therefore reduced function(Im et al., 2008). The anterior cingulate is part of the limbic system and is involved in functions such as attention, reward-based learning, executive function (decision making, problem solving), impulse control, social interactions, emotional regulation and empathy, many of which are known to be affected in children with heavy PAE(Mattson et al., 2011). Our finding is strikingly similar to that reported by Migliorini et al. (2015), who showed that the right caudal anterior cingulate surface area was significantly reduced in adolescents with heavy PAE, a finding associated with significantly poorer ability for impulse control (Migliorini et al., 2015). Others have shown that cortical surface area may be more sensitive to the effects of PAE than cortical thickness(Gross et al., 2018). Contrary to our findings, a study investigating low-moderate PAE reported *larger* surface areas throughout many cortical regions(Lees et al., 2020). Of interest in our study, PAE throughout gestation, rather than in the first trimester only, appeared to influence cortical morphology, consistent with the concept that brain effects depend on both dose and timing of PAE(Guerri, 1998). It is worthy of note, however, that socio-economic factors may have confounded our results. After adjusting for prenatal binge-level alcohol exposure before pregnancy recognition, as well as socio-economic factors that differed between PAE groups, the volume and area differences we found in the caudal anterior cingulate cortex diminished.

4.4. WM microstructure and macrostructure

After adjusting for ICV, the PAE T1-T3 group exhibited lower FC in fibres of the right cingulum bundle compared with controls. Fibres affected were concentrated in the anterior cingulate and mid-cingulate regions of the dorsal cingulum(Bubb et al., 2018). This finding suggests that children with PAE throughout pregnancy may have a lower total cross-sectional area of the dorsal cingulum, and thus the possibility of a reduced capacity for information transfer between the cingulate gyrus and other frontal, parietal and parahippocampal regions, compared with those with no PAE. In line with our findings, altered structural and functional connectivity of the cingulum was reported in a study focusing on default mode network dysfunction in adults with PAE

(Santhanam et al., 2011). Also, poorer diffusion microstructure of the cingulum has been reported in children with PAE(Paolozza et al., 2017), including with FASD(Lebel et al., 2008; Sowell et al., 2008). The dorsal cingulum is involved with emotion, motivation, executive function including attention, pain, and possibly memory(Bubb et al., 2018). Many of these functions are poorer in children with heavy PAE(Mattson et al., 2011), which may be partially explained by alterations to the cingulum bundle. It was interesting to note that both the volume and surface area of the right anterior cingulate and its white matter connections (right dorsal cingulum bundle) were altered in the PAE T1-T3 group compared with controls. White matter tracts follow four waves of development with association tracts such as the cingulum not appearing until the second trimester(Horgos et al., 2020). Most AQUA study mothers dramatically reduced their alcohol intake upon pregnancy recognition even if they continued to drink(Muggli et al., 2022b), which suggests that this network may be particularly vulnerable to the teratogenic effects of even small amounts of alcohol.

The PAE T1 group had higher FC than controls in the cerebellar WM and brainstem, and the PAE T1-T3 group had higher FC than controls in the anterior limb of the internal capsule and genu of the corpus callosum before adjusting for ICV. However, these differences did not remain after adjusting for ICV, indicating they were not independent of brain size differences. Neither did these findings reflect the direction we would expect, and therefore may not be robust. Other studies report cerebellar disturbances in those with PAE, including cerebellar hypoplasia(Boronat et al., 2017), size reductions(Sullivan et al., 2020; Zhou et al., 2018) and microstructural abnormalities(Ghazi Sherbaf et al., 2019; Wozniak and Muetzel, 2011), and lower fractional anisotropy has been reported in the anterior limb of the internal capsule in children with FASD(Stephen et al., 2021). Conversely, higher fractional anisotropy in the genu of the corpus callosum has been reported in children with PAE(Kar et al., 2021), a finding consistent with ours.

4.5. Strengths and limitations

AQUA is one of the most rigorous studies of the effects of low-moderate and binge drinking in the world. It utilized a novel assessment of alcohol consumption, developed with input from consumers (Muggli et al., 2015), and incorporated timing, frequency and quantity. A large representative community cohort of pregnant women was recruited, and rich information on important mediators and confounders of outcome were collected(Muggli et al., 2014). Thus, we were able to adjust for potential confounding for those socio-demographic factors that differed between the PAE groups. The larger AQUA study collected very detailed information on dose, frequency and timing of maternal alcohol consumption, and identified 6 groupings for PAE, including abstained, low discontinued (trimester 1), moderate discontinued, low sustained, moderate sustained, and high sustained (Muggli et al., 2022b), however we did not have sufficient power in the sub-sample with MRI to investigate these finer-scale groups. We did, however, account for children of women who were binge drinkers by adjusting for this variable in our analyses.

This study used Freesurfer brain volumes, previously shown to be accurate in a pre-adolescent PAE cohort when compared with manual tracing(Biffen et al., 2020). We also utilized advanced diffusion MRI analysis, fixel-based analysis, which overcomes many limitations of the commonly used DTI model, meaning our results may be more sensitive and biologically meaningful. Nevertheless, our results should be interpreted with caution considering all the group differences found in this study were either present before or after adjusting for ICV, not both, and therefore may not be considered entirely robust. Typically, we would consider a brain region to be specifically vulnerable only if any differences found were independent of overall brain size differences, which is the purpose of adjusting for ICV. Furthermore, despite adjusting statistically for multiple comparisons, it is possible these findings may have type 1 error, particularly given three different cortical and fixel-based measures were utilized.

Future investigations will correlate brain imaging findings, including further multimodal advanced imaging analyses, with 3-D analysis of craniofacial shape and neuropsychological measures to further detect any subtle effects of low-moderate PAE on the brain. This will help to determine the interplay between important markers of PAE and to detect patterns of brain changes associated with low-moderate PAE that predict outcomes.

4.6. Conclusion and implications

In conclusion, few structural brain alterations were identified in 6- to 8-year-olds with low-moderate PAE, particularly when pertinent socio-economic factors were considered. After accounting for ICV, low-moderate PAE *throughout gestation* was associated with a smaller right caudal anterior cingulate cortex volume and surface area, and a smaller cross-sectional area of the right cingulum bundle compared with the no PAE group. These findings illustrate possible consequences of low-moderate PAE throughout gestation on specific brain structures and concur with previous literature examining children with heavy PAE (Lebel et al., 2008; Migliorini et al., 2015; Sowell et al., 2008). Such brain alterations may help explain some of the impairments common to children exposed to PAE(Mattson et al., 2011). However, caution must be taken when interpreting these results, as when we accounted for socio-economic factors that differed between our PAE groups, the strength of many group differences diminished. Thus, future studies are required in this very important area to better inform messages provided by health professionals to the public regarding harms of alcohol use in pregnancy.

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CRediT authorship contribution statement

Deanne K. Thompson: Writing – original draft, Visualization, Funding acquisition, Formal analysis, Conceptualization. **Claire E. Kelly:** Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Thijs Dhollander:** Writing – review & editing, Formal analysis, Data curation. **Evelyne Muggli:** Writing – review & editing, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Stephen Hearps:** Writing – review & editing, Validation, Funding acquisition, Conceptualization. **Sharon Lewis:** Writing – review & editing, Funding acquisition, Conceptualization. **Thi-Nhu-Ngoc Nguyen:** Investigation, Data curation. **Alicia Spittle:** Writing – review & editing, Funding acquisition, Conceptualization. **Elizabeth J. Elliott:** Writing – review & editing, Funding acquisition, Conceptualization. **Anthony Penington:** Writing – review & editing, Funding acquisition, Conceptualization. **Jane Halliday:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Peter J. Anderson:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

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