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Prenatal methadone exposure is associated with altered neonatal brain development

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ABSTRACT

Methadone is used for medication-assisted treatment of heroin addiction during pregnancy. The neurodevelopmental outcome of children with prenatal methadone exposure can be sub-optimal. We tested the hypothesis that brain development is altered among newborn infants whose mothers were prescribed methadone.

20 methadone-exposed neonates born after 37 weeks' postmenstrual age (PMA) and 20 non-exposed controls underwent diffusion MRI at mean PMA of 39^{+2} and 41^{+1} weeks, respectively. An age-optimized Tract-based Spatial Statistics (TBSS) pipeline was used to perform voxel-wise statistical comparison of fractional anisotropy (FA) data between exposed and non-exposed neonates.

Methadone-exposed neonates had decreased FA within the centrum semiovale, inferior longitudinal fasciculi (ILF) and the internal and external capsules after adjustment for GA at MRI ($p < 0.05$, TFCE corrected). Median FA across the white matter skeleton was 12% lower among methadone-exposed infants. Mean head circumference (HC) z-scores were lower in the methadone-exposed group (-0.52 (0.99) *vs* 1.15 (0.84), $p < 0.001$); after adjustment for HC z-scores, differences in FA remained in the anterior and posterior limbs of the internal capsule and the ILF. Polydrug use among cases was common.

Prenatal methadone exposure is associated with microstructural alteration in major white matter tracts, which is present at birth and is independent of head growth. Although the findings cannot be attributed to methadone *per se*, the data indicate that further research to determine optimal management of opioid use disorder during pregnancy is required. Future studies should evaluate childhood outcomes including infant brain development and long-term neurocognitive function.

1. Introduction

Globally, in 2015 there were estimated to be 17.7million past-year users of heroin or opium, and increased heroin use is a major driver of the current opioid epidemic (World Drug Report, 2017). Pregnant women with opioid use disorder (OUD) due to heroin are recommended medication-assisted treatment (MAT) with an alternative opioid (usually methadone or buprenorphine) because treatment is associated with improved use of antenatal services, reduced use of heroin during pregnancy and reduced preterm delivery. Fetal benefits of MAT include improved growth and lower risk of intrauterine death (American College of Obstetricians and Gynecologists, 2017).

Methadone is a synthetic long acting μ-opioid agonist, which crosses the placenta freely, thereby exposing the developing fetus to exogenous opioid at a critical period of brain development. Pre-clinical studies suggest that prenatal methadone exposure may modify developing dopaminergic, cholinergic and serotonergic systems, and alter myelination. Antenatal exposure to the drug has behavioral consequences including depression, anxiety, and impaired learning, memory and social function (Chen et al., 2015; Robinson et al., 1996; Vestal-Laborde et al., 2014; Wong et al., 2014). In humans, prenatal methadone exposure is associated with increased incidence and severity of neonatal abstinence syndrome (NAS) (Wilson et al., 1981; Zelson et al., 1973) compared with heroin exposure, and with altered visual maturation in

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childhood (McGlone et al., 2013a; McGlone et al., 2008; Whitham et al., 2010). These observations raise the possibility that prenatal methadone exposure may modify early brain development; however, the possible role of confounding by postnatal events, including pharmacotherapy with opioid for NAS and environmental factors, leaves uncertainty about the impact of prenatal methadone exposure on the developing brain.

Diffusion MRI (dMRI) is an established technique for studying brain development in early life. It provides objective measures of white matter microstructure that are sensitive to atypical developmental and injurious processes in the perinatal period, and which correlate with neurodevelopmental outcome in childhood (Counsell et al., 2014). Specifically, fractional anisotropy (FA) is a voxel-wise measure of the directional dependence of water molecule diffusion in tissue which is influenced by fiber density, axonal diameter and myelination, thereby enabling inference about underlying tissue microstructure. Tract-based Spatial Statistics (TBSS) enables unbiased group-wise analysis of FA volumes derived from dMRI data (Ball et al., 2010; Smith et al., 2006). It has been applied to neonatal dMRI to map microstructural change in white matter tracts of preterm infants at term equivalent age (Anjari et al., 2007), to identify clinical risk factors for altered brain development (Anblagan et al., 2016; Ball et al., 2010; Boardman et al., 2014), and to investigate neuroprotective treatment strategies in randomized clinical trials (Azzopardi et al., 2016; O'Gorman et al., 2015; Porter et al., 2010).

Based on the harmful effects of prenatal methadone exposure on neural systems and abnormal behavioral outcomes in pre-clinical models; and on human studies which suggest a modifying effect of prenatal methadone on postnatal behavior and development, we hypothesized that white matter development of neonates would be altered in neonates exposed to methadone *in utero*. We used TBSS to examine risks associated with prenatal methadone exposure, while minimizing the role of confounding by postnatal events and drug exposures.

2. Methods and materials

2.1. Participants

The study was conducted according to the principles of the Declaration of Helsinki and ethical approval was obtained from the UK National Ethics Service (South East Scotland Research Ethics Committee 02, 14/SS/1106). Written informed parental consent was obtained for all participants. The study group consisted of infants > 37 weeks' postmenstrual age (PMA) whose mothers had been prescribed methadone during pregnancy for the treatment of OUD (cases) and a comparator group of healthy infants born at > 37 weeks' PMA whose mothers did not use opioids (controls).

Mothers of cases were identified through a specialist antenatal clinic for pregnant women with substance misuse. All cases were born at the Royal Infirmary of Edinburgh between February 2015 and April 2017 and underwent MRI brain scanning at the Clinical Research Imaging Centre, University of Edinburgh. The controls were selected, based on age matching, from a previously described group of healthy term neonates recruited as part of a study of typical brain development (Blesa et al., 2016) (South East Scotland Research Ethics Committee 02, 13/SS/0143). For cases and controls, exclusion criteria were congenital infection or chromosomal abnormalities, or any implanted medical device.

Clinical and demographic information was extracted from the mother and infant clinical records. Birth weight and head circumference (HC) were described in terms of z-score for week of gestational age, calculated using INTERGROWTH-21st reference standards (Villar et al., 2014). The Scottish Index of Multiple Deprivation (SIMD) was used to characterize deprivation. The SIMD is the official Government tool used to identify areas of deprivation: it divides Scotland into around 6505 areas each containing around 350 households and assigns an index to each area based on multiple measures of deprivation. The data are ranked from most to least deprived and are presented as deciles.

2.2. Ascertainment of maternal drug use

Details of methadone use, tobacco smoking, alcohol intake, and use of non-prescribed drugs were ascertained from medical records (including prescription charts), biological screening samples when these were performed as part of clinical care, and maternal interview at the time of delivery (V.M.)

2.3. MRI acquisition

MRI was performed on a Siemens Magnetom Verio 3T system (Siemens Healthcare Gmbh, Erlangen, Germany) using a 12-channel matrix phased array head coil. All infants were scanned axially to acquire: 3D T1-weighted MPRAGE volume (1 mm^3 resolution), T2-weighted STIR (0.9 mm^3 resolution), T2-weighted FLAIR (1 mm^3 resolution), and diffusion MRI (dMRI) (11 T2- and 64 diffusion encoding direction (b = 750 s/mm^2) single-shot spin-echo echo planar imaging (EPI) volumes with 2 mm isotropic voxels, TE = 106 ms and TR = 7300 ms. Images were reported by a pediatric radiologist with experience in neonatal MRI (AQ), according to the system described by Woodward et al. (Woodward et al., 2006), with the modification for grey matter scores proposed by Leuchter et al. (Leuchter et al., 2014).

MRI was performed in the neonatal period during natural sleep, without sedation. A neonatologist was present for the duration of each MRI scan, and the infant had continuous oxygen saturation and heart rate monitoring. For acoustic protection, flexible earplugs and neonatal earmuffs (MiniMuffs, Nat's Medical Inc., CA) were used.

2.4. Tract-based spatial statistics

DMRI data were preprocessed using FSL tools (FMRIB, Oxford, UK; http://www.ndcn.ox.ac.uk/divisions/fmrib). This included brain extraction, and removal of bulk infant motion and eddy current induced artefacts by registering the diffusion-weighted volumes to the first T2-weighted EPI volume for each subject. Using DTIFIT, FA volumes were generated for every subject. Diffusion volumes were assessed visually and were excluded if there was motion corruption.

TBSS analysis was performed using a pipeline optimized for neonatal dMRI data (Ball et al., 2010). An average FA volume and mean FA skeleton (thresholded at FA > 0.15) were created from the aligned data. Statistical comparison between groups with and without exposure to methadone during pregnancy was performed with FSL's Randomise using a general linear univariate model, with GA at image acquisition and HC z-score at image acquisition listed as covariates. All FA data were subject to family-wise error correction for multiple comparisons following threshold-free cluster enhancement (TFCE) and are shown at $p < 0.05$ (Smith and Nichols, 2009).

2.5. Statistics

Student's *t*-test or the Mann-Whitney test was used to investigate differences in clinical and demographic variables between infants exposed to methadone (n = 20) and those not exposed (n = 20) and chi-squared or Fisher's exact test was used to compare proportions. Statistical analysis was performed using SPSS v22.0 (SPSS Inc., Chicago, IL).

3. Results

3.1. Participants

Conventional structural and dMRI data amenable to TBSS analysis were acquired from 40 neonates: 20 cases (10 female), who were