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ABSTRACT

Introduction Preterm birth is closely associated with altered brain development and is a leading cause of neurodevelopmental, cognitive and behavioural impairments across the life course. We aimed to investigate neuroanatomic variation and adverse outcomes associated with preterm birth by studying a cohort of preterm infants and controls born at term using brain MRI linked to biosamples and clinical, environmental and neuropsychological data.

Methods and analysis Theirworld Edinburgh Birth Cohort is a prospective longitudinal cohort study at the University of Edinburgh. We plan to recruit 300 infants born at <33 weeks of gestational age (GA) and 100 healthy control infants born after 37 weeks of GA. Multiple domains are assessed: maternal and infant clinical and demographic information; placental histology; immunoregulatory and trophic proteins in umbilical cord and neonatal blood; brain macrostructure and microstructure from structural and diffusion MRI (dMRI); DNA methylation; hypothalamic–pituitary–adrenal axis activity; social cognition, attention and processing speed from eye tracking during infancy and childhood; neurodevelopment; gut and respiratory microbiota; susceptibility to viral infections; and participant experience. Main analyses include creation of novel methods for extracting information from neonatal structural and dMRI, regression analyses of predictors of brain maldevelopment and neurocognitive outcome associated with preterm birth, and determination of the quantitative predictive performance of MRI and other early life factors for childhood outcome.

Ethics and dissemination Ethical approval has been obtained from the National Research Ethics Service (NRES), South East Scotland Research Ethics Committee (NRES numbers 11/55/0061 and 13/SS/0143 (phase I) and 16/SS/0154 (phase II)), and NHS Lothian Research and Development (2016/0255). Results are disseminated through open access journals, scientific meetings, social media, newsletters anda study website (www.tebc.ed.ac.uk), and we engage with the University of Edinburgh public relations and media office to ensure maximum publicity and benefit.

Strengths and limitations of this study

► Three hundred preterm infants and a comparator group of 100 term controls are studied longitudinally from before birth to school age.
► Phenotypical information includes data from brain MRI, biosamples, participant report, direct observation and clinical data from maternal and infant medical records.
► We collected data about a range of theoretically informed variables to understand the impact of preterm birth on everyday lives of families.
► A data access and collaboration policy sets out the terms and conditions on which deidentified data are available to the research community.
► Participants are recruited from a single centre.

INTRODUCTION

Preterm delivery is estimated to affect 10.6% of all live births around the world, which equates to 14.84 million births per annum.[1] In resource-rich settings, advances in perinatal care and service delivery have led to improved survival over the past two decades: around 30% of infants born at 22 weeks who are offered stabilisation at birth will survive, and this number increases to around 80% for births at 26 weeks.[2–5] However, early exposure to extrauterine life can impact brain development and is closely associated with long-term intellectual disability, cerebral palsy, autism spectrum disorder, attention deficit hyperactivity disorder, psychiatric disease, and problems with language, behaviour and socioemotional function (for review, see Johnson and Marlow[6]). There are no treatments that reduce risk of impairment, which extends across the life course and carries considerable personal cost to affected individuals, and high health and education costs to society.[7]

Little is known about the ontogenesis of neurocognitive and psychiatric problems associated with preterm birth, or the biological, environmental and social risk factors associated with susceptibility and resilience. Much information about the cerebral effects

of preterm birth comes from historic cohorts that do not reflect modern perinatal care practices; studies have been cross-sectional with outcomes assessed in very early childhood before important cognitive and social functions emerge; conventional diagnostic tools for assessing neurodevelopment are imprecise; and cohorts linked to imaging and biological metadata are few, so mechanisms are poorly understood. There is an unmet need to study a contemporary cohort of preterm infants that is comprehensively characterised from genes to anatomy to function, integrated with information about the social graph.

Our aims were, first, to build a longitudinal cohort of preterm infants and term controls that is phenotyped with brain imaging and biological information to investigate causal pathways to, and consequences of, atypical brain development and injury; second, to develop novel computational algorithms for mapping brain growth and connectivity in early life; third, to identify new and multifactorial methods for early detection of children at risk of long-term impairment; and fourth, to identify early life biological and environmental risk and resilience factors that affect the developing brain and so pave the way for new therapeutic strategies.

METHODS AND ANALYSIS
Study design
This is a single-centre prospective longitudinal cohort study.

Study setting
The Theirworld Edinburgh Birth Cohort (TEBC) study is conducted at the University of Edinburgh and the Simpson Centre for Reproductive Health (SCRH), which is located at the Royal Infirmary of Edinburgh, NHS Lothian, UK. The SCRH provides maternity and newborn services for residents of the City of Edinburgh and the Lothians. It receives 7000 deliveries per annum and is the regional centre for all neonatal intensive care in South East Scotland. Approximately 100 infants with birthweight of <1500 g receive intensive care at SCRH per annum.

Participant recruitment, initial assessment and data collection points 1–3 (table 1) take place in the SCRH or the Edinburgh Imaging Facility, Royal Infirmary of Edinburgh. Follow-up assessments take place in a dedicated child development laboratory at the University of Edinburgh, through online and in-person completion of questionnaires, and in neonatal outpatient clinics at the SCRH (time points 4–7, table 1). Recruitment began in November 2016 and is planned to be completed in 2021.

Study participants
Inclusion criteria
Cases: 300 preterm infants born at <33 weeks of gestational age (GA)*
 Controls: 100 term infants born at >37 weeks of GA*
 *GA is estimated based on first trimester ultrasound.

Cases are included if a mother booked her pregnancy and delivered at SCRH (the study centre), or if a mother booked her pregnancy at a hospital outside the study centre but was transferred to it with her baby in utero due to planned or expected birth <33 weeks. Preterm infants who are transferred to SCRH ex utero for intensive care are not included.

Exclusion criteria
1. Infants with congenital anomalies: structural or functional anomalies (eg, metabolic disorders) that occur during intrauterine life and can be identified prenatally, at birth or later in life (WHO definition).
2. Infants with a contraindication to MRI at 3T.

Sample selection and recruitment
Sample size
A key aim of the study was to investigate causes and consequences of preterm brain injury/atypical development by analysing data about brain macrostructure and microstructure from structural and quantitative MRI with biological, environmental and neuropsychological outcome data. In the absence of established methodology for power calculations using quantitative MRI techniques, the sample size is based on: exemplars of indicative sensitivity and power from computational modelling and previous data; and realistic assessment of recruitment, successful image acquisition of 85%, and follow-up. Studies indicate it is possible to detect groupwise differences in brain anatomy associated with specific exposures by applying computational techniques to MRI data from relatively small group sizes in univariate models; for example, tract-based spatial statistics and network-based statistics are sensitive to generalised changes in microstructure and connectivity with 20–60 infants per group,[8–14] and morphometric methods detect anatomical variation with similar group sizes, depending on the image feature of interest.[15 16] However, a key strength of the study is that larger samples (n=300–400) are required to construct multivariate models (needed to investigate multiple exposures that influence brain development), to combine information from different MRI modalities using data-driven methods, to investigate associations between image phenotypes and behavioural outcomes which often require larger study populations,[17 18] and to develop analytical methods that support causal inference. Another aim was the development of novel computational methods for mapping growth and connectivity in development. While certain technical developments such as image segmentation and methods for studying crossing fibres are achievable with sample sizes of <100,[19–22] larger sample sizes are needed to address other challenges. For example, larger atlases of the developing brain than are currently available are required to understand population diversity, and machine learning methods are being used to develop image biomarkers and to improve the interoperability of multisite acquisitions, which will enable researchers to increase study power, carry out essential

Table 1 Schedule of assessments, data collection methods, sample type/domain, and the test or task

Time point	Age	Data collection method	Sample type/domain of measurement	Test/task
1	Antenatal	Records and interview	Socioeconomic status	Maternal and paternal education, Scottish Index of Multiple Deprivation derived from home postcode
			Medical/demographic	Family and medical history and exposures
2	Birth	Records, questionnaire and tissue	Medical	History and exposures
				Anthropometry
			Placenta	Structured histopathology rating and storage
			Cord blood	Panel of immunoregulatory and trophic proteins
				Gene expression array*
3	Neonatal	Tissue: blood	Blood spot	Panel of immunoregulatory and trophic proteins
				Gene expression array*
		Tissue: saliva	Epigenetics	DNA methylation
		Tissue: nasal swab	Nasal lining fluid	Antimicrobial peptides including cathelicidin levels*
			DNA/RNA	Respiratory microbiota*
		Stool	DNA/RNA	Gut microbiota*
		Direct observation	Medical	Anthropometry
			Retinopathy of prematurity assessment	Grade retinopathy
			Parent IQ	National Adult Reading Test
		MRI	Brain structure and connectivity	Structural and diffusion 3T MRI
		Questionnaire	Medical/demographic	Breast feeding and updated perinatal medical history
				Edinburgh Postnatal Depression Scale
				Parenting Daily Hassles
				WHO—Quality Of Life
				Adult Temperament Questionnaire
4	4.5 months	Questionnaire, by post or online or phone interview	Demographics	Updated socioeconomic status, maternal education, breastfeeding/nutrition activities
			Infant temperament	Infant Behaviour Questionnaire, Revised, short form
				Edinburgh Postnatal Depression Scale
			Parent well-being	WHO—Quality Of Life
		Tissue: nasal swab	DNA/RNA	Respiratory microbiota*

Continued

Table 1 Continued

Time point	Age	Data collection method	Sample type/domain of measurement	Test/task
5	9 months	Tissue: saliva	Epigenetics	DNA methylation
			HPAA	Cortisol: waking, 30 min after waking, before bed
				Prestill-face and poststill-face procedure
		Tissue: nasal swab	Nasal lining fluid	Antimicrobial peptides including cathelicidin levels*
			DNA/RNA	Respiratory microbiota*
		Eye tracking	Social development	Free scanning: neutral faces
				Free scanning: pop-out task, looking to faces and distractors
				Free scanning: social preferential looking to social and non-social images
				Free scanning: dancing ladies social and non-social videos
			Attention	Switching and disengagement: gap-overlap task, fixation to central and peripheral cues
				Sustained attention: follow the bird task, following moving target
			Processing speed	Free scanning: odd-one-out visual search task (simple letters version)
				Free-scanning: word–picture matching task
		Direct observation	Visual acuity	Keeler card assessment
			Social development	Still-face procedure (sub-set with computational motor assessment)
				Parent–child play, for later behavioural coding: (subset with computational motor assessment)
		Questionnaire	Infant temperament	Infant Behaviour Questionnaire, Revised, short form
				Sleep and Settle Questionnaire
			Language	MacArthur Communicative Development Inventory (words and gestures)
			Parent well-being	WHO—Quality Of Life
			Feedback	Feedback form, monitoring satisfaction with research project
		Direct observation	Anthropometry	Growth
		Parent interview	Demographics	Family circumstances update form including breastfeeding, socioeconomic status (home postcode)
			Developmental level	Vineland Adaptive Behaviour Scales: comprehensive interview form

Continued

Table 1 Continued

Time point	Age	Data collection method	Sample type/domain of measurement	Test/task
6	2 years	Direct observation	Ophthalmology	Refraction
			Anthropometry	Growth
		Tissue: nasal swab	Nasal lining fluid	Antimicrobial peptides including cathelicidin levels*
			DNA/RNA	Respiratory microbiota*
		Eye tracking	Social development	Free scanning: neutral faces
				Free scanning: pop-out task, looking to faces and distractors
				Free scanning: social preferential looking to social and non-social images
				Free scanning: dancing ladies social and non-social videos
				Switching and disengagement: gap-overlap task, fixation to central and peripheral cues
			Attention	Sustained attention: follow the bird task, following moving target
			Processing speed	Free scanning: odd-one-out visual search task
				Free-scanning: word–picture matching task
		Direct observation	Social development	Parent–child play, for later behavioural coding
			Executive function	Following Instructions task
			Bayley-III	General developmental level*
		Questionnaire	Temperament	Early Childhood Behaviour Questionnaire, Revised, short form
				Child Sleep Habits Questionnaire
			Language	MacArthur Communicative Development Inventory (words and sentences)
			Social development	Quantitative Checklist for Autism in Toddlers
			Executive function	BRIEF-P
				Early Executive Function Questionnaire
			Developmental level	Vineland Adaptive Behaviour Scales: comprehensive parent rating form
			Parent well-being	WHO—Quality Of Life
			Feedback	Feedback form, monitoring satisfaction with research project
		Parent interview	Demographics	Family circumstances update form including breastfeeding, socioeconomic status (home postcode)

Continued

Table 1 Continued

Time point	Age	Data collection method	Sample type/domain of measurement	Test/task
7	5 years	Tissue: saliva	Epigenetics	DNA methylation
			HPAA	Cortisol
		Tissue: nasal swab	DNA/RNA	Respiratory microbiota*
		Direct observation	Anthropometry	Growth
			Blood pressure	Hypertension
			Ophthalmology	Refraction and acuity
			Social development	Parent–child play, for later behavioural coding
			Executive function	Following Instructions task
			Developmental level	Mullen Scales of Early Learning
		Eye tracking	Social development	Free scanning: neutral faces
				Free scanning: pop-out task, looking to faces and distractors
				Free scanning: social preferential looking to social and non-social images
				Free scanning: dancing ladies social and non-social videos
			Attention	Switching and disengagement: gap-overlap task, fixation to central and peripheral cues
				Sustained attention: follow the bird task, following moving target
			Processing speed	Free scanning: odd-one-out visual search task (complex objects version)
		Questionnaire	Temperament	Strengths and Difficulties Questionnaire (both teacher and parent report versions)
			Language	Children's Communication Checklist
			Social development	Social Communication Questionnaire: Current
			Executive function	DuPaul ADHD Rating Scale
				BRIEF-P
			Visual perception	Cerebral Visual Impairment Inventory
			Parent well-being	WHO—Quality Of Life
			Feedback	Feedback form monitoring satisfaction with research project
			Developmental level	Vineland Adaptive Behaviour Scales: domain-level parent rating form
		Parent interview	Demographics	Family circumstances update form including socioeconomic status (home postcode)

*Subset of participants.
ADHD, attention deficit hyperactivity disorder; BRIEF-P, Behaviour Rating Inventory for Executive Function, Preschool; HPAA, hypothalamic–pituitary–adrenal axis.

replication studies and investigate risk and resilience in brain development conferred by the genome.[23–25] We expect to address some of these issues with the planned sample of 400 and to make material contribution to wider data-sharing initiatives subject to the study's data access and collaboration policy.

Identifying participants

Cases: infants born to women who present to the SCRH with threatened preterm labour and for whom delivery is planned or expected at less than 33 weeks GA.

Controls: infants born to women who attend the SCRH and deliver at >37 weeks of GA.

The protocol reported here was partially developed through a separate, pilot 'phase I' cohort of 150 cases and 40 controls. This phase I pilot included neonatal MRI and infant eye tracking, and a subset of this group is now participating in the 5-year assessment as described here (time point 7, table 1).

Screening for eligibility

The research nurse/clinical research fellow identifies potential participants using maternity TrakCare, which is a system used by maternity services throughout NHS Lothian to record information about pregnancies and maternal care, and the neonatal electronic patient record. The clinical team provides an introductory leaflet about TEBC to eligible parents and then informs the research team of parents who wish to discuss the study in greater detail. Those parents meet with a member of the research team and are provided with the participant information sheet.

Participants from phase I studies being recalled for time point 7 (at 5 years) are contacted by the research team using contact details provided previously. Study information (introductory letter, patient information sheet, reply slip and prepaid envelope) is sent by post and followed up with a telephone call to answer any questions and to review willingness to participate.

Consenting participants

Informed written consent is sought in two stages: first, consent for perinatal and neonatal sampling and assessment at initial enrolment to the study; second, consent for assessments postdischarge to 5 years is taken at time point 3 (see table 1 below).

For phase I participants being recalled, consent is taken at the recall appointment, following circulation and discussion of the content by post and phone, as described previously.

Informed consent may only be taken by a member of the research team with training in International Council for Harmonisation—Good Clinical Practice and procedures for research involving children and young people.

Coenrolment

The SCRH is an academic perinatal medicine centre that hosts observational research studies, and it is a recruiting centre for randomised controlled trials of therapies designed to improve the outcome of preterm infants and their mothers. Parents/carers of TEBC participants are encouraged to consider entry into such studies if eligible. Coenrolment is informed by 'Guidelines for Co-enrolment', produced by the Academic and Clinical Central Office for Research and Development (ACCORD), which is a partnership between the University of Edinburgh and NHS Lothian Health Board. Coenrolment will be recorded.

Cohort retention

Participants and their families are kept up to date with research progress through newsletters, Twitter, Facebook and a website (www.tebc.ed.ac.uk). Birthday cards are sent to participants and we hold an annual event for research updates and public outreach.

Withdrawal of study participants

The decision to withdraw from the study is either at parental/carer request or at the request of the attending consultant physician or the principal investigator for clinical reasons.

Outcomes and data analysis

Table 1 summarises the assessment schedule, data collection methods, sample type/domain, and the test or task. Data from cases and controls are collected using the same data collection instruments.

Maternal and infant clinical and demographic information

Data are abstracted from the mothers' and infants' electronic medical records onto a standardised data collection sheet. A structured maternal interview is used to collect additional information that may not be recorded in routinely collected data, for example, detailed family history about neurodevelopmental and mental health problems, and over-the-counter prescription and recreational drugs taken during pregnancy. For deaths, the cause and postmortem findings will be recorded.

Placentas

After delivery, placentae from all preterm infants are formalin fixed and stored at 4°C before sampling. The placentae are sampled according to a standardised protocol; distal and proximal sections of cord (the proximal section being taken at 1.5 cm from above the fetal surface), a roll of extraplacental membranes starting at the point of rupture and four full thickness sections from each quadrant. All are stained with H&E and reported using a standardised, structured approach that describes any pathological features present, including but not limited to, fetal thrombotic vasculopathy, villitis, chorioamnionitis, funisitis and features of uteroplacental ischaemia.[12 26]

Immunoregulatory and trophic proteins

Analysis of a panel of immunoregulatory and trophic proteins (interleukin (IL)-1b, IL-2, IL-4, IL-5, IL-6, IL-8, IL-12, IL-17, tumour necrosis factor (TNF)-α, macrophage

inflammatory protein (MIP)-1b, brain-derived neurotrophic factor (BDNF), granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-10, IL-18, interferon (IFN)-g, TNF-β, monocyte chemoattractant protein (MCP)-1, MIP-1a, C3, C5a, C9, matrix metalloproteinase (MMP)-9, regulated on activation, normal T cell expressed and secreted (RANTES) and C reactive protein (CRP)) is undertaken on umbilical cord and neonatal blood samples. These proteins are selected to offer information with respect to the proinflammatory and anti-inflammatory innate responses, as well as the adaptive immune response. Blood is collected using Schleicher and Schuell 903 filter paper (6.0×3.2 mm spots per subject) and analysed using a multiplex immunoassay (Meso Scale Discovery) at Statens Serum Institute, Copenhagen. We use the approach described by Skogstrand *et al*[27] to analyse differences in concentration between cases and controls.

Structural and dMRI
A Siemens MAGNETOM Prisma 3T MRI clinical scanner (Siemens Healthcare, Erlangen, Germany) and 16-channel phased-array paediatric head receive coil are used to acquire three-dimensional (3D) T1-weighted (T1w) magnetization-prepared rapid acquisition with gradient echo (MPRAGE) structural volume scan (acquired voxel size=1 mm isotropic) with inversion time (TI) 1100 ms, echo time (TE) 4.69 ms and repetition time (TR) 1970 ms; a 3D T2-weighted (T2w) sampling perfection with application-optimized contrasts by using flip angle evolution (SPACE) structural scan (voxel size=1 mm isotropic) with TE 409 ms and TR 3200 ms; and a multishell axial dMRI scan (16×b=0 s/mm^2, 3×b=200 s/mm^2, 6×b=500 s/mm^2, 64×b=750 s/mm^2, 64×b=2500 s/mm^2) with optimal angular coverage[28] (online supplementary materials 1–3). If the infant stays settled, axial 3D susceptibility-weighted imaging (TR=28 ms, TE=20 ms, 0.75×0.75×3.0 mm acquired resolution) and axial 2D fluid-attenuated inversion-recovery BLADE imaging (TR=10 000 ms, TE=130 ms, TI=2606 ms, 0.94×0.94×3.0 mm acquired resolution) are acquired. In a subgroup of participants, magnetisation transfer saturation imaging is acquired for evaluation of tissue myelin content, consisting of three sagittal 3D multiecho spoiled gradient echo scans (TE=(1.54, 4.55 and 8.56 ms), 2 mm isotropic acquired resolution), magnetisation-transfer, proton density-weighted (TR=75 ms, flip angle =5°) and T1w (TR=15 ms, flip angle =14°) acquisitions (see online supplementary material 4). Tissue heating and acoustic noise exposure are limited throughout the examination through the use of active noise cancellation and by setting the gradient slew rate and other pulse sequence parameters appropriately. Participants are scanned in normal mode with respect to both tissue heating and peripheral nerve stimulation.

Conventional images are reported by a paediatric radiologist using a structured system.[29 30] We use image data to generate novel processing techniques optimised for neonatal data,[11 19–21 31] and we will use these and other publicly available pipelines for processing neonatal data[13 32 33] to derive image features for analyses with collateral data relating to exposures and outcomes. These include, but are not limited to, tract-based, morphometric and structural connectivity analyses.[10–12 34–38]

DNA storage
DNA is extracted from saliva, stored and catalogued at the Edinburgh Clinical Research Facility, ready for downstream analyses.

DNA methylation
Saliva is sampled using the DNA OG-575 kit (DNAGenotek, Ottawa, Ontario, Canada). DNA extraction is performed using published methods,[36] and DNA methylation (DNAm) analyses are carried out at the Genetics Core of the Edinburgh Clinical Research Facility (Edinburgh, UK) using Illumina Infinium MethylationEPIC (San Diego, California, USA), with interrogation of the arrays against ~850 k methylation sites. We will investigate perinatal influences on DNAm using principal component analysis, mediation and correlation analyses.

Hypothalamic–pituitary–adrenal axis (HPAA)
Salivary cortisol is used as a marker of HPAA activity. Saliva is collected in Sarstedt tubes at specified times at 9 months and 5 years. Timed saliva samples are also collected during the 9-month appointment before and after a behavioural paradigm (still face), which is known to elicit a biological stress response (one sample pretest and two samples posttest to capture reaction and recovery). Samples are stored at −20°C and analysed in batches at each time point. Anthropometric data are recorded at 9 months and 2 and 5 years, and blood pressure is measured at 5 years

Eye tracking
We record eye movements in response to visual stimuli at 9 months, 2 years and 5 years using a Tobii x60 eye tracker and bespoke analysis software (MATLAB). Images are presented on a display monitor with a resolution of 1440×900 pixels. The Tobii×60 system tracks both eyes to a rated accuracy of 0.3 degrees at a rate of 60 Hz. We analyse looking patterns, including time to first fixate and looking time at areas of interest, in tasks designed to enable inference about social development, attention and processing speed.[35 39]

Standardised assessments
Standardised assessments of neurodevelopment by direct observation at appropriate time points are Bayley-III scales, Mullen Scales of Early Learning (MSEL) and parental IQ (National Adult Reading Test). We selected the MSEL for assessing cognitive ability at 5 years because it has separate verbal and non-verbal standardised scores, so it is useful for assessing cognitive abilities in children with social communication and language difficulties; internal consistency reliability and test/retest reliability for the five component scales is high; and the early learning composite (and its components) correlate with other psychometric tests used in this age group. We will use validated questionnaires to

assess: infant/parent temperament; parent/family characteristics (postnatal depression, stress, quality of life and socioeconomic status); infant/child sleep habits; language development; social development; executive functions; cerebral visual impairment; medical diagnoses; and behavioural outcomes (parent and teacher ratings). We also record parent–child interaction for subsequent analysis via video coding of complex behaviours in a naturalistic context.

Susceptibility to viral infection

We collect unstimulated nasal secretion samples (nasosorption samples) using methods described by Thwaites et al.[40] This collection is brief, minimally invasive and a minimally distressing process. Nasosorption nasal lining fluid is collected using Nasosorption Fxi synthetic absorption matrix strips inserted into the anterior part of the inferior turbinate of the nasal cavity. After 30s of absorption, the strip is removed, capped, maintained at 4°C for up to 4hours and then frozen at −80°C. From these nasal fluid samples we will assess the levels of antimicrobial peptides, including cathelicidin, and inflammatory cytokines by ELISA or luminex assay. Collection of these at birth (term equivalent age), 9 months and 2 years will enable us to characterise birth levels, levels at time points significant for respiratory syncytial virus (RSV) infection/disease and at a later time point.

Respiratory and gut microbiota

We collect faecal and nasopharyngeal swabs (paediatric Copan e-swab with flocked nylon fibre tip) as has been described in the WHO guideline for respiratory sampling of bacterial pathogens.[41] Faecal material and e-swabs (in RNA protect) are frozen at −80°C until further analyses. DNA and RNA will be extracted[42] and metagenomics analyses will be executed by 16S-based sequencing according to previously described methods.[43] We will study temporal relationships between preterm birth and early life characteristics, consecutive microbiota development, inflammation and methylation findings, and respiratory and neurocognitive developmental outcomes.

Computational motor assessment

Lightweight, wearable, wireless motion sensors are deployed to record the movement of a subset of infants at 9 months during the still-face paradigm and parent–child interaction. Data are anonymised before being securely transferred to the University of Strathclyde for analysis. These data will be analysed to test for differences in motor function between at-risk and low-risk infants, and will employ machine learning algorithms to detect patterns predictive of developmental outcome at 2 and 5 years, and their potential for clinical stratification across the neurodevelopmental disorders and psychometric profiles (IQ, adaptive function and language). Further, motor data at 9 months can be correlated against neuroanatomical features measured by MRI scan at birth and developmental scales at 9 months.

Patient and public involvement

We seek feedback from parents/carers to monitor satisfaction with research participation at 9 months and 2 and 5 years, and we have a public facing website that describes results from the study.

ETHICS AND DISSEMINATION

Ethical approval has been obtained from the National Research Ethics Service (NRES), South East Scotland Research Ethics Committee (NRES numbers 11/55/0061 and 13/SS/0143 (phase I) and 16/SS/0154 (phase II)), and NHS Lothian Research and Development (2016/0255). Results are disseminated through open access journals, scientific meetings, social media, newsletters and a study website (www.tebc.ed.ac.uk), and we engage with the University of Edinburgh public relations and media office to ensure maximum publicity and benefit.

Safety assessment

There are no safety issues associated with collection of: placental tissue, umbilical cord/neonatal blood, saliva, faeces or hair. There are no safety issues in the conduct of planned neuropsychological assessments.

MRI does not involve ionising radiation and there are no known risks from MRI provided standard safety measures for 3T scanning are in place. Infants are fed and wrapped and allowed to sleep naturally in the scanner. Pulse oximetry, electrocardiography and temperature are monitored. Flexible earplugs and neonatal earmuffs (MiniMuffs, Natus) are used for acoustic protection. All scans are supervised by a doctor or nurse trained in neonatal resuscitation. The scan is interrupted if there are any abnormalities in monitoring or if the baby wakes.

It is possible that incidental findings may be found on MRI or from questionnaires, for example, intracranial structural anomalies or postnatal depression, respectively. In these circumstances, the findings are discussed with the participant's parent, and referral to the appropriate NHS service is made.

The study is run by a management group that includes the principal investigator, a minimum of two coinvestigators, the study coordinator and administrative and financial officers. A delegation log details the responsibilities of each member of staff working on the study. A scientific advisory board oversees the conduct and progress of the study. The study is cosponsored by the University of Edinburgh and NHS Lothian ACCORD.

Publication and data statement

The principles set down by the International Committee of Medical Journal Editors for authorship and non-author contributors are followed for publications and presentations resulting from the study. A data access and collaboration policy sets out the terms and conditions on which deidentified TEBC data, stimuli and tasks are accessible to the research community following reasonable request (www.tebc.ed.ac.uk).

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REFERENCES

1. Chawanpaiboon S, Vogel JP, Moller A-B, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *Lancet Glob Health* 2019;7:e37–46.
2. Mehler K, Oberthuer A, Keller T, et al. Survival among infants born at 22 or 23 weeks' gestation following active prenatal and postnatal care. *JAMA Pediatr* 2016;170:671–7.
3. Patel RM, Rysavy MA, Bell EF, et al. Survival of infants born at Periviable gestational ages. *Clin Perinatol* 2017;44:287–303.
4. Norman M, Hallberg B, Abrahamsson T, et al. Association between year of birth and 1-year survival among extremely preterm infants in Sweden during 2004-2007 and 2014-2016. *JAMA* 2019;321:1188–99.
5. Myrhaug HT, Brurberg KG, Hov L, et al. Survival and impairment of extremely premature infants: a meta-analysis. *Pediatrics* 2019;143:e20180933.
6. Johnson S, Marlow N. Early and long-term outcome of infants born extremely preterm. *Arch Dis Child* 2017;102:97–102.
7. Mangham LJ, Petrou S, Doyle LW, et al. The cost of preterm birth throughout childhood in England and Wales. *Pediatrics* 2009;123:e312–27.
8. Porter EJ, Counsell SJ, Edwards AD, et al. Tract-based spatial statistics of magnetic resonance images to assess disease and treatment effects in perinatal asphyxial encephalopathy. *Pediatr Res* 2010;68:205–9.
9. Ball G, Boardman JP, Arichi T, et al. Testing the sensitivity of Tract-Based spatial statistics to simulated treatment effects in preterm neonates. *PLoS One* 2013;8:e67706.
10. Monnelly VJ, Anblagan D, Quigley A, et al. Prenatal methadone exposure is associated with altered neonatal brain development. *Neuroimage Clin* 2018;18:9–14.
11. Blesa M, Sullivan G, Anblagan D, et al. Early breast milk exposure modifies brain connectivity in preterm infants. *Neuroimage* 2019;184:431–9.
12. Anblagan D, Pataky R, Evans MJ, et al. Association between preterm brain injury and exposure to chorioamnionitis during fetal life. *Sci Rep* 2016;6:37932.
13. Ball G, Counsell SJ, Anjari M, et al. An optimised tract-based spatial statistics protocol for neonates: applications to prematurity and chronic lung disease. *Neuroimage* 2010;53:94–102.
14. Jakab A, Ruegger C, Bucher HU, et al. Network based statistics reveals trophic and neuroprotective effect of early high dose erythropoietin on brain connectivity in very preterm infants. *Neuroimage Clin* 2019;22:101806.
15. Boardman JP, Counsell SJ, Rueckert D, et al. Early growth in brain volume is preserved in the majority of preterm infants. *Ann Neurol* 2007;62:185–92.
16. Inder TE, Warfield SK, Wang H, et al. Abnormal cerebral structure is present at term in premature infants. *Pediatrics* 2005;115:286–94.
17. Boardman JP, Counsell SJ. Invited review: factors associated with atypical brain development in preterm infants: insights from magnetic resonance imaging. *Neuropathol Appl Neurobiol* 2019;3. doi:10.1111/nan.12589. [Epub ahead of print: 20 Nov 2019].
18. Ball G, Aljabar P, Nongena P, et al. Multimodal image analysis of clinical influences on preterm brain development. *Ann Neurol* 2017;82:233–46.
19. Serag A, Wilkinson AG, Telford EJ, et al. SEGMA: an automatic segmentation approach for human brain MRI using sliding window and random forests. *Front Neuroinform* 2017;11:2.
20. Blesa M, Serag A, Wilkinson AG, et al. Parcellation of the healthy neonatal brain into 107 regions using atlas propagation through intermediate time points in childhood. *Front Neurosci* 2016;10:220.
21. Serag A, Blesa M, Moore EJ, et al. Accurate learning with few atlases (alfa): an algorithm for MRI neonatal brain extraction and comparison with 11 publicly available methods. *Sci Rep* 2016;6:23470.
22. Pecheva D, Tournier J-D, Pietsch M, et al. Fixel-based analysis of the preterm brain: disentangling bundle-specific white matter microstructural and macrostructural changes in relation to clinical risk factors. *Neuroimage Clin* 2019;23:101820.
23. Dickie DA, Shenkin SD, Anblagan D, et al. Whole brain magnetic resonance image atlases: a systematic review of existing atlases and caveats for use in population imaging. *Front Neuroinform* 2017;11:1.
24. Job DE, Dickie DA, Rodriguez D, et al. A brain imaging Repository of normal structural MRI across the life course: brain images of normal subjects (brains). *Neuroimage* 2017;144:299–304.
25. Shenkin SD, Pernet C, Nichols TE, et al. Improving data availability for brain image biobanking in healthy subjects: practice-based suggestions from an international multidisciplinary Working group. *Neuroimage* 2017;153:399–409.
26. Redline RW, Faye-Petersen O, Heller D, et al. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. *Pediatr Dev Pathol* 2003;6:435–48.
27. Skogstrand K, Thorsen P, Nørgaard-Pedersen B, et al. Simultaneous measurement of 25 inflammatory markers and neurotrophins in neonatal dried blood spots by immunoassay with xMAP technology. *Clin Chem* 2005;51:1854–66.
28. Caruyer E, Lenglet C, Sapiro G, et al. Design of multishell sampling schemes with uniform coverage in diffusion MRI. *Magn Reson Med* 2013;69:1534–40.
29. Woodward LJ, Anderson PJ, Austin NC, et al. Neonatal MRI to predict neurodevelopmental outcomes in preterm infants. *N Engl J Med* 2006;355:685–94.
30. Leuchter RH-V, Gui L, Poncet A, et al. Association between early administration of high-dose erythropoietin in preterm infants and brain MRI abnormality at term-equivalent age. *JAMA* 2014;312:817–24.
31. Anblagan D, Bastin ME, Sparrow S, et al. Tract shape modeling detects changes associated with preterm birth and neuroprotective treatment effects. *Neuroimage Clin* 2015;8:51–8.
32. Smith SM, Jenkinson M, Johansen-Berg H, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *Neuroimage* 2006;31:1487–505.
33. Makropoulos A, Robinson EC, Schuh A, et al. The developing human connectome project: a minimal processing pipeline for neonatal cortical surface reconstruction. *Neuroimage* 2018;173:88–112.

34 Boardman JP, Walley A, Ball G, *et al*. Common genetic variants and risk of brain injury after preterm birth. *Pediatrics* 2014;133:e1655–63.

35 Telford EJ, Fletcher-Watson S, Gillespie-Smith K, *et al*. Preterm birth is associated with atypical social orienting in infancy detected using eye tracking. *J Child Psychol Psychiatry* 2016;57:861–8.

36 Sparrow S, Manning JR, Cartier J, *et al*. Epigenomic profiling of preterm infants reveals DNA methylation differences at sites associated with neural function. *Transl Psychiatry* 2016;6:e716.

37 Krishnan ML, Van Steenwinckel J, Schang A-L, *et al*. Integrative genomics of microglia implicates DLG4 (PSD95) in the white matter development of preterm infants. *Nat Commun* 2017;8:428.

38 Telford EJ, Cox SR, Fletcher-Watson S, *et al*. A latent measure explains substantial variance in white matter microstructure across the newborn human brain. *Brain Struct Funct* 2017;222:4023–33.

39 Gillespie-Smith K, Boardman JP, Murray IC, *et al*. Multiple measures of fixation on social content in infancy: evidence for a single social cognitive construct? *Infancy* 2016;21:241–57.

40 Thwaites RS, Jarvis HC, Singh N, *et al*. Absorption of nasal and bronchial fluids: precision sampling of the human respiratory mucosa and laboratory processing of samples. *J Vis Exp* 2018;131. doi:10.3791/56413. [Epub ahead of print: 21 Jan 2018].

41 Satzke C, Turner P, Virolainen-Julkunen A, *et al*. Standard method for detecting upper respiratory carriage of Streptococcus pneumoniae: updated recommendations from the world Health organization pneumococcal carriage Working group. *Vaccine* 2013;32:165–79.

42 de Steenhuijsen Piters WAA, Heinonen S, Hasrat R, *et al*. Nasopharyngeal microbiota, host transcriptome, and disease severity in children with respiratory syncytial virus infection. *Am J Respir Crit Care Med* 2016;194:1104–15.

43 Bosch AATM, de Steenhuijsen Piters WAA, van Houten MA, *et al*. Maturation of the infant respiratory microbiota, environmental drivers, and health consequences. A prospective cohort study. *Am J Respir Crit Care Med* 2017;196:1582–90.