


# BMJ Open Prediction of childhood brain outcomes in infants born preterm using neonatal MRI and concurrent clinical biomarkers (PREBO-6): study protocol for a prospective cohort study

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## ABSTRACT

**Introduction** Infants born very preterm are at risk of adverse neurodevelopmental outcomes, including cognitive deficits, motor impairments and cerebral palsy. Earlier identification enables targeted early interventions to be implemented with the aim of improving outcomes.

**Methods and analysis** Protocol for 6-year follow-up of two cohorts of infants born <31 weeks gestational age (PPREMO: Prediction of Preterm Motor Outcomes; PREBO: Prediction of Preterm Brain Outcomes) and a small term-born reference sample in Brisbane, Australia. Both preterm cohorts underwent very early MRI and concurrent clinical assessment at 32 and 40 weeks postmenstrual age (PMA) and were followed up at 3, 12 and 24 months corrected age (CA). This study will perform MRI and electroencephalography (EEG). Primary outcomes include the Movement Assessment Battery for Children second edition and Full-Scale IQ score from the Wechsler Intelligence Scale for Children fifth edition (WISC-V). Secondary outcomes include the Gross Motor Function Classification System for children with cerebral palsy; executive function (Behavior Rating Inventory of Executive Function second edition, WISC-V Digit Span and Picture Span, Wisconsin Card Sorting Test 64 Card Version); attention (Test of Everyday Attention for Children second edition); language (Clinical Evaluation of Language Fundamentals fifth edition), academic achievement (Woodcock Johnson IV Tests of Achievement); mental health and quality of life (Development and Well-Being Assessment, Autism Spectrum Quotient-10 Items Child version and Child Health Utility-9D).

## Aims

1. Examine the ability of early neonatal MRI, EEG and concurrent clinical measures at 32 weeks PMA to predict motor, cognitive, language, academic achievement and mental health outcomes at 6 years CA.
2. Determine if early brain abnormalities persist and are evident on brain MRI at 6 years CA and the relationship to EEG and concurrent motor, cognitive, language, academic achievement and mental health outcomes.

## Strengths and limitations of this study

- Prediction of Preterm Brain Outcomes (PREBO)-6 is the first and to date only prospective cohort study of infants born very preterm with early (32 weeks postmenstrual age) structural and advanced neuroimaging in Australia and one of only a few worldwide.
- PREBO-6 is the only cohort study of infants born very preterm with comprehensive concurrent clinical correlates of the early brain MRI.
- The neuroimaging follow-up protocol at 6 years corrected age is innovative in its inclusion of structural, advanced diffusion and functional MRI and electroencephalography to comprehensively evaluate brain macrostructure, microstructure and functional maturation.
- The neurodevelopmental follow-up protocol, carefully designed to minimise risk of fatigue impacting quality of the data collected, includes a comprehensive evaluation of academic achievement and mental health, in addition to motor and cognitive outcomes, ensuring that the true predictive accuracy of early brain MRI can be fully established.
- The potential limitations of this study include the lack of an adequate full-term comparison group and the use of normative data as reference, the nature of psychiatric measures instead of the gold standard psychiatric interview and the motion artefacts inherent in early MRI.

**Ethics and dissemination** Ethical approval has been obtained from Human Research Ethics Committees at Children's Health Queensland (HREC/19/QCHQ/49800) and The University of Queensland (2019000426). Study findings will be presented at national and international conferences and published in peer-reviewed journals.  
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**Web address of trial** <http://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?ACTRN=12619000155190p>

## INTRODUCTION

### Background and rationale

Infants born preterm experience a range of adverse neurodevelopmental outcomes including cognitive, behavioural, educational and motor impairments with 5%–10% developing cerebral palsy (CP).<sup>1–3</sup> Early accurate identification of those at risk of adverse outcomes enables prognostication of outcomes, initiation of targeted early interventions and provision of family psychological and financial supports. The relationship between MRI at term-equivalent age (TEA; 40–42 weeks postmenstrual age) and childhood neurodevelopmental outcomes of motor, cognitive, language, behaviour and school achievement has been demonstrated.<sup>4–6</sup> In addition to replicating these findings, the aim of this study is to evaluate whether clinical assessments, MRI and electroencephalography (EEG) performed even earlier in the neonatal period (in this case at 30–32 weeks postmenstrual age) predict outcomes at school age; and whether predictive ability is increased by combining early MRI and clinical measures of motor, neurological and neurobehavioural function. Earlier neonatal MRI and clinical assessment open a new window for therapeutic interventions at a time of rapid brain development while the infant is still in the neonatal intensive care unit. Increasingly, very preterm infants are discharged prior to TEA, so an earlier MRI ensures that infants who are at greatest risk of adverse outcomes are identified and receive appropriate surveillance and early interventions.

Our prior work established two internationally unique prospective cohorts (PPREMO: Prediction of Preterm Motor Outcomes; PREBO: Prediction of Preterm Brain Outcomes) of 269 infants born <31 weeks gestation with MRI and concurrent clinical assessment of motor, neurological and neurobehavioural function performed at 30–32 weeks postmenstrual age (PMA; ‘early’) and again at TEA. Infants were followed until 2 years corrected age (CA) to determine cognitive and motor outcomes and CP. We demonstrated identification of infants at risk of adverse motor outcomes and CP at 2 years CA using early MRI and clinical biomarkers.<sup>7–9</sup> To our knowledge, the PREBO cohort is now one of four worldwide with >100 very preterm infants with early MRI and neurodevelopmental outcomes, and the only study with MRI at 3T.

Obtaining detailed motor, cognitive, academic achievement and mental health outcomes data in early childhood (during the critical time period of transition to school) will facilitate the development of diagnostic techniques for the infants at risk of adverse neurodevelopmental outcomes using very early MRI, EEG and clinical biomarkers. This is essential to inform prognosis, service provision and the need for targeted interventions maximising resource allocation.

### Study objectives

Using information from the follow-up of two prospective cohorts of infants born at <31 weeks gestation, this study aims to:

1. Examine the ability of early neonatal MRI, EEG and concurrent clinical measures at 32 weeks PMA to predict motor, cognitive, language, academic achievement and mental health outcomes at 6 years CA.

Hypothesis 1: there is a predictive relationship between assessments of early MRI, EEG and clinical measures at 30–32 weeks PMA and motor, cognitive, language, academic achievement and mental health outcomes at 6 years CA, in infants born very preterm.

2. Determine if early brain abnormalities persist and are evident on brain MRI at 6 years CA and if they are related to EEG and concurrent motor, cognitive, language, academic achievement and mental health outcomes at 6 years CA.

Hypothesis 2: early and TEA brain morphometry and microstructural abnormalities predict changes evident on MRI at 6 years CA in infants born very preterm.

Hypothesis 3: structural and advanced diffusion biomarkers obtained from MRI at 6 years CA are related to EEG and concurrent motor, cognitive, language, academic achievement and mental health at 6 years CA.

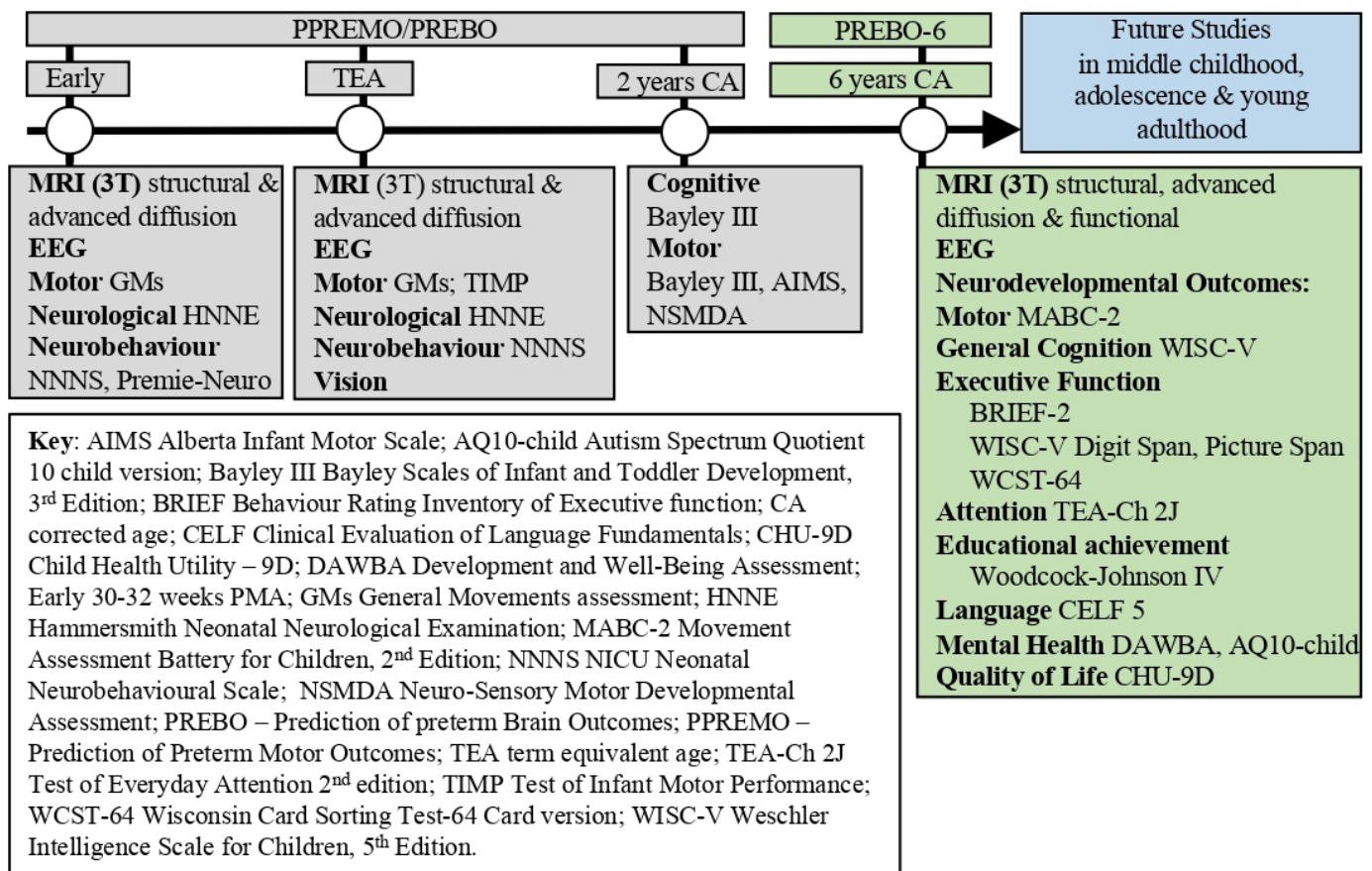
## METHODS AND ANALYSIS

### Study design and setting

This prospective observational cohort study of infants born very preterm with a small comparison group of infants born at term, will be conducted at the Centre for Children’s Health Research (CCHR) and the Herston Imaging Research Facility (HIRF) in Brisbane, Australia. The study will start in May 2019 and run for 5 years. Children will be assessed at 6 years CA rather than chronological age, based on evidence of significant differences in neurodevelopmental outcomes scores between the two ages for very preterm born children when assessed in childhood.<sup>10–13</sup>

### Inclusion criteria

- ▶ Preterm cohort: children who participated in the original PPREMO<sup>14</sup> or PREBO studies and turn 6 years CA within the study period. Children were recruited from the tertiary academic hospital, The Royal Brisbane and Women’s Hospital (RBWH) in Brisbane, Australia. Enrolment dates were as follows: PPREMO January 2013 to February 2016; PREBO: February 2016 to December 2018. Inclusion criteria included a gestational age at birth <31 weeks, from English-speaking families living within 200 km of the hospital. Children were ineligible if they had any congenital or chromosomal abnormality likely to impact their neurodevelopmental outcome or if they had contraindications to MRI. No infants were excluded a priori due to medical fragility or high ventilatory requirements, however a small number of infants who were enrolled in the study were unable to progress in the study if they were too unwell or medically unstable and unable to undergo MRI and clinical assessment before 35+6 weeks PMA.



**Figure 1** Flow chart of prospective cohort study (blue represents planned future studies; grey represents data already collected in this cohort; green represents protocol for this 6-year stage of the cohort study).

► Term born reference cohort: a small comparison group of children were recruited from the postnatal ward of the RBWH or as interested volunteers by word of mouth, between February 2013 and May 2015 in the PPREMO study.<sup>14</sup> Children were eligible if they were born between 38 and 41 weeks PMA following an uncomplicated pregnancy and delivery, had a birth weight above the 10th percentile and were not admitted to neonatal intensive or special care units following their birth. No children with a history of maternal diabetes, hypertension, hypothyroidism, chorioamnionitis or any other pregnancy or delivery complications were included. As part of the early study protocol, all infants underwent neurological assessment which confirmed a lack of neurological abnormalities in the cohort.

### Study regimen

Study procedures are depicted in [figure 1](#). The child and their parent/guardian will be invited to attend a one-off assessment that will be conducted over 2 days at the CCHR and HIRF. Day 1 will include the clinical assessments, EEG and mock MRI/MRI preparation, The MRI will be conducted on day 2. Experienced clinical researchers will perform the motor and neurodevelopmental assessments at the first visit, and all personnel involved in data

collection will be blinded to clinical history, MRI and early (birth to 2 years) MRI and clinical assessment results. All MRI and EEG technicians will be blinded to 6-year clinical assessment results, clinical history, early MRI and early (birth to 2 years) clinical assessment results. The study protocol has been carefully designed to minimise the risk of fatigue. Specifically, for the neurodevelopmental protocol, the order of assessments has been finalised after taking into consideration the feedback from pilot testing as well as the length of administration, cognitive demands of the assessment and task monotony for children aged 6 years. Adequate rest breaks are also provided across different assessment blocks.

### Assessments

#### Primary outcomes

##### Motor function

Children's motor abilities will be assessed using the standardised and norm-referenced Movement Assessment Battery for Children, second edition (MABC-2).<sup>15</sup> Subscales include manual dexterity, aiming and catching and balance. This tool has been designed to identify motor impairments in children aged 3–16 years and is widely considered the gold standard test for motor performance in children born preterm.<sup>16</sup>

### General cognition

Children's general cognitive development will be assessed using the Full-Scale IQ score derived from the seven subtests of the Wechsler Intelligence Scale for Children, fifth edition: Australian and New Zealand Standardised Edition (WISC-V A&NZ).<sup>17</sup> These subtests span five core domains of verbal comprehension, visual-spatial ability, fluid reasoning, working memory and processing speed. Subtests include similarities, vocabulary, block design, matrix reasoning, figure weights, digit span and coding. The WISC-V is the latest edition of the gold standard Wechsler scales with updated psychometric properties and normative data for the Australian population, and has been shown to be a robust screener for general cognitive deficits.<sup>17</sup>

### Secondary outcomes

#### Functional severity of cerebral palsy

Children with CP will be classified for functional severity using the Gross Motor Function Classification System (GMFCS) by two trained physiotherapists. The GMFCS has internationally established validity, reliability and stability for the classification and prediction of motor function of children with CP aged 2–12 years.<sup>18</sup> The 6–12 years descriptions from the extended and revised GMFCS will be used.<sup>19</sup> It has an acceptable inter-rater and intra-rater (test–retest) reliability (generalisability coefficients 0.93 and 0.68, respectively).<sup>18</sup>

#### Executive function

Manifestations of everyday executive function will be assessed using the parent-rated Behavior Rating Inventory of Executive Function, second edition (BRIEF-2).<sup>20</sup> This 63-item rating scale is one of the most widely used behavioural measures of executive function in clinical practice and epidemiological research,<sup>21</sup> with outcomes extending across the domains of behavioural, emotional and cognitive regulation. The BRIEF-2 has strong internal consistency ( $r=0.76$ – $0.97$  for parent report), and moderate-to-strong concurrent validity ( $r=0.33$ – $0.67$  with measures of attention deficit hyperactivity disorder and behaviour problems).<sup>22</sup>

Two components of working memory, verbal and visual memory, will be assessed using complementary norm-referenced measures. Verbal working memory will be assessed using the Digit Span of the WISC-V A&NZ.<sup>17</sup> This involves repeating a string of numbers presented verbally to the children with increasing complexity from two digits to eight in the same order as presented as well as backwards (ie, repeat the number string in the reverse order, if '3-7-2' the child should say '2-7-3'). Visual working memory will be assessed using the Picture Span of the WISC-V A&NZ<sup>17</sup> that involves memorising pictures and identifying them in order on subsequent pages, with increasing span complexity like the Digit Span subtest.<sup>23</sup>

Cognitive flexibility (and inhibitory control) will be assessed using the Wisconsin Card Sorting Test-64 Card version (WCST-64).<sup>24</sup> This test requires the child to

adjust the sorting criterion of a set of cards depending on 'correct' or 'incorrect' feedback provided by the examiner. The total number of perseverative errors, that is, persistence of following an old sorting criterion when the rule has noticeably changed, provides a reliable marker of cognitive flexibility, whereas non-perseverative errors provide a measure of inhibitory control. Moderate-to-good reliability coefficients have been reported ( $r=0.37$ – $0.72$ ) for the WCST-64.

#### Attention

Children's selective and sustained attention will be assessed using the Test of Everyday Attention for Children, second edition.<sup>25</sup> The current edition is shorter than the original test, developed for an efficient evaluation of attention abilities in younger children. This test has been shown to have reliable psychometric properties in relation to existing measures of attention as well as being comparable to the earlier version which has been widely used across multiple cohort studies of preterm children.<sup>25</sup>

#### Language

Children's language abilities will be assessed using six subtests of the Clinical Evaluation of Language Fundamentals, fifth edition, a criterion-referenced assessment of language skills in children aged 5–21 years with normative data available for the Australian population.<sup>26</sup> Subtests include: word structure, word classes, formulated sentences, recalling sentences, following directions and sentence comprehension, and will provide core language along with receptive and expressive language index scores.<sup>26</sup> Good reliability (internal consistency) and validity (content, convergent and divergent) statistics have been reported.<sup>26</sup>

#### Educational achievement

The Australian adaptation of the Woodcock-Johnson Test of Achievement, fourth edition will assess children's emerging abilities of educational and academic attainment across the domains of reading and math.<sup>27</sup> Reading abilities will be assessed using the broad reading cluster comprising letter-word identification, sentence reading fluency and passage comprehension subtests. Math abilities will be assessed using the broad math cluster comprising calculation, math facts fluency and applied problems subtests. These tests have strong psychometric properties and correlate well with the Kaufman Test of Educational Achievement and Wechsler Achievement Test.<sup>27</sup>

#### Mental health

The Development and Well-Being Assessment, a semi-structured online child psychiatric interview will be used for a comprehensive evaluation of the risk of Diagnostic and Statistical Manual, fifth edition (DSM-5) psychiatric disorders.<sup>28</sup> This assessment consists of a three-step process. First, parents complete an online psychiatric interview, which is then screened by a computer algorithm

to predict the likely diagnosis of a DSM-5 disorder. This computer-generated risk profile is then reviewed by a clinician who provides the final diagnosis. This tool has excellent psychometric properties in relation to diagnoses based on clinical interviews and has been recently used across three large and internationally renowned preterm studies from Australia,<sup>29</sup> the UK<sup>28 30</sup> and New Zealand.<sup>31</sup>

All children will be assessed for risk of autism using the Autism Spectrum Quotient-Child (AQ10-child), a 10-item parent-report screening measure for children aged 4–11 years.<sup>32</sup> This screening measure was developed from the Quantitative Checklist for Autism in Toddlers and has reliable psychometric properties.

The Child Health Utility-9D is a generic questionnaire for assessing the quality of life in early school age children. An algorithm calculates a single preference-based utility index for health states (giving a single generic preference-based indicator of each individual's health state).<sup>33</sup>

#### *Brain structural integrity and connectivity (MRI and EEG)*

Brain MRI will be performed using a 3T Siemens Prisma with 64-channel head coil at HIRF. Participants will be familiarised with the MRI procedures before the scan. During the MRI, the child will watch an age-appropriate movie of their choice, except during the acquisition of the functional MRI (fMRI). Structural brain images will be acquired using high-resolution three-dimensional (3D) T1-weighted (T1w) MPRAGE and 3D T2-weighted (T2w) SPACE from the Adolescent Brain Cognitive Dataset study,<sup>34</sup> and a high-resolution 3D T2-weighted FLAIR. Diffusion MRI data will be acquired using a multishell approach with 20 directions at  $b=1000\text{ s/mm}^2$  and 60 directions at  $b=3000\text{ s/mm}^2$ . fMRI data will be acquired, one using a block design with a simple hand-tapping task, and one while the children are at rest (resting state fMRI ~5 min). Full detail of MRI sequence parameters is included in online supplementary file 1, and a summary table of key MRI parameters is included as online supplementary file 2. These sequences will allow the investigation of (i) brain structure (volumetry, cortical thickness, white matter lesions), (ii) myelin mapping and brain microstructure (fibre density and organisation) and (iii) connectivity (both structural and functional). The total scan time will be 45 min. We have high compliance rates (>90%) for 3T MRI at this age in >200 children.

#### *Magnetic resonance imaging*

All MRI data will be preprocessed to reduce the impact of head motion and image artefacts, and spatially normalised into a standard space.

Structural MRI—segmentations of the three cerebral tissues (white matter, grey matter, cerebrospinal fluid), as well as specific anatomical regions (eg, subregions of the cerebellum, subcortical grey matter, lateral ventricles and parcellations of the cortical regions) will be obtained using FreeSurfer,<sup>35</sup> producing global and local measures of tissue volume for statistical analysis.<sup>36</sup> Measures of

cortical shape will be extracted from these segmentations, including measures of cortical thickness, cortical curvature and sulcal depth, which will be aggregated by cortical region in the statistical analysis.<sup>37</sup> Using both T1w and T2w FLAIR MR sequences, white matter lesions will be extracted, with lesions volume will be aggregated based on ROIs in the International Consortium of Brain Mapping white matter atlas, for statistical analysis. Semi-quantitative methods for scoring the structural MRI data will be used, potentially using a novel scale for classifying structural brain injury in childhood.<sup>38</sup> This scale has been validated on a cohort of children and adolescents aged 5–18 years with CP, however will need to be validated and shown to be reliable in preterm cohorts with lower rates of CP.<sup>39</sup> These analyses will enable the validation of structural findings at early, TEA and 6 years CA time points.<sup>8 38 40</sup>

Diffusion MRI—maps of fractional anisotropy and mean diffusivity will be calculated using MRtrix3,<sup>41</sup> and maps of NODDI (neurite orientation dispersion and density imaging) measures (intracellular volume fraction, isotropic volume fraction, orientation dispersion) will be calculated using AMICO (accelerated microstructure imaging via convex optimisation).<sup>42</sup> Fibre orientation distributions will be estimated using multishell multi-tissue constrained spherical deconvolution (MRtrix3), and fixel measures (fibre density, fibre-bundle cross-section, fibre density and bundle cross-section) will be calculated.<sup>43</sup> Anatomically constrained tractography will be performed using MRtrix3,<sup>44</sup> and filtered using Spherical Deconvolution Informed Filtering of Tractograms.<sup>45</sup> Summary measures of diffusion metrics will be extracted from regions and tracts of interest for statistical analysis.

fMRI—a generalised linear model (GLM) will be used to analyse the block design motor task and resting state fMRI data, accounting for confounding factors (MRI drift, temporal correlations), using the Statistical Parametric Mapping software.<sup>46</sup> The GLM provides a t-statistic for each voxel, and accounting for multiple comparisons using Random Field Theory, produce maps of statistically significant regions of activation. Resting state fMRI data will be analysed using Independent Component Analysis using the FMRIB Software Library to identify functional networks, to generate structural and microstructural measures in these networks for statistical analysis.<sup>47</sup>

#### *Electroencephalography*

Dense array EEG will be collected at 6 years CA as done for the cohort at Early and TEA time points. A child-friendly, high-density 128 channel EEG cap (Geodesic EGI Hydrocel GSN 130) will be used to record EEG in a quiet room, while the child is awake, seated in a comfortable chair. Each child's EEG will be recorded over a 20 min period, with 5 min for EEG cap preparation and EEG data recorded with an allotted 5 min 'eyes-open' (EEG recorded with normal blinking) and 5 min 'eyes-closed' period, respectively. EEG acquisition will be sampled at 2048 Hz.<sup>14</sup> Following acquisition, EEG datasets will be

preprocessed to generate artefact-free EEG and analysed using standard methods by power spectral density to quantify brain activity at distinct frequencies<sup>48</sup> and whole-brain connectivity measures will be subsequently employed.

### Sample size

The sample size is determined by the number of participants in the PPREMO<sup>14</sup> or PREBO studies who will turn 6 years CA within the 5-year study period 2019–2024 (n=178 preterm; n=18 term controls; total n=196). For both aims, we can detect significant associations between MRI measures (early MRI and MRI at 6 years CA) with clinical scores at 6 years CA ( $r>0.2$ ), with  $>80\%$  power and  $\alpha=0.05$ . This is consistent with previous studies of similar-sized cohorts, which found early MRI measures (abnormality scores and white matter microstructure) associated with childhood outcomes with a similar effect size.<sup>5 6</sup> If we conservatively assume 15% attrition to 6 years, we will still be able to detect  $r>0.22$ . For group-wise comparisons, assuming documented variability in MABC-2<sup>49</sup> (mean=9.2, SD=2.4) and IQ<sup>50</sup> (mean=97, SD=17), we are able to detect a difference between children with brain injury (n=59, assuming a 30% rate of mild to severe injury based on the brain abnormality MRI scores measured at early MRI<sup>8</sup> to those without any observable injury (n=137), as small as 1.1 points on the MABC-2 and 7.5 IQ points, with  $\alpha=0.05$  and  $>80\%$  power. Assuming 15% attrition these detectable differences increase to 1.2 and 8.1 points. This assumes MRI measurement error to be significantly less than observed anatomical variability, which has been observed on a similarly aged cohort.<sup>51</sup>

### Statistical analysis

For aims 1 and 2, predictive regression models will be constructed. Explanatory variables include: (i) brain morphometry, microstructure and clinical measures at 30–32 weeks or TEA (aim 1), (ii) brain morphometry, microstructure and clinical measures at 6 years CA (aim 2).

For the primary analysis, data from the 30–32 weeks postmenstrual age or TEA time point will be associated with CP diagnosis, motor or cognitive outcome using standard regression models. Secondary analyses would associate the same explanatory variables with other outcomes, as well as include data from multiple time points (including 6 years CA), using mixed-effects models that take into account within-child correlation. Variables modelling the interaction between brain structure and child's age will be included to account for potential longitudinal changes in brain anatomy. Linear regression will be used for continuous outcomes (eg, WISC-V); logistic regression for binary outcomes (eg, CP/no CP) and multinomial logistic regression for categorical outcomes with  $>2$  categories (eg, Woodcock-Johnson). Initially, univariable associations between candidate predictor variables and outcomes will be

investigated. Variables likely to be relevant in predicting response and potentially included in the model based on a priori expectation include perinatal variables, patient age, gender and socioeconomic status. Socioeconomic status was measured at birth and is defined using a score of six aspects of social status. These include family structure, education of primary caregiver, occupation of primary income earner, employment status of primary income earner, language spoken at home and maternal age.<sup>14</sup> Each of the items is scored from 0 to 2 for a maximum total score of 12, with higher scores indicating a higher level of social risk. A score of 2 or above is considered as high social risk and has been validated in cohorts of preterm born children.<sup>52–54</sup> Missing MRI-derived variables can be addressed using imputation to avoid potential bias, while missing patient covariates or clinical outcomes will be removed from the analysis. Multivariable analysis will determine the most appropriate combination of predictors. For each multivariable model a list of candidate variables will be selected and tested univariably. Variables that are univariably significant at the  $p<0.2$  level will be considered for potential inclusion in the multivariable model, which will be constructed using a stepwise feature selection/elimination procedure based on standard criteria (Akaike Information Criterion; Bayesian Information Criterion). Results will be presented as effect estimates and 95% CIs. Model calibration will be tested graphically and using Hosmer-Lemeshow  $\chi^2$  statistic. Internal validation will be performed using bootstrap resampling via 10-fold cross-validation. Model bias due to overfitting will be estimated and the final model corrected accordingly.

### Data availability statement

The study team are available to collaborate with other research teams on receipt of a reasonable request to access study data. Expressions of interest to access study data, made out to the corresponding author, will be considered and then group level or individual level deidentified data could be shared as appropriate.

### Patient and public involvement

Author RJ on this protocol paper is our consumer representative. She is the parent of a premature child born at 26 weeks gestational age, with early brain injury and subsequent CP as well as a healthy term born infant who participated in this study in the term born reference group. She has been part of the study team since the inception of this 6-year follow-up stage of the cohort study, participated in funding applications, contributed to development of the study protocol and assessment of burden to families, provided feedback on wording of Parent Information documentation and report document templates for families and continues to join study meetings for ongoing evaluation of study progress from a patient and family perspective.

## Sources of bias

There are potential sources of bias in the present study. These may include the lack of an adequately representative full-term comparison group and reliance on published normative data. Furthermore, the selection of psychiatric measures, despite their robust psychometric properties in relation to the clinical psychiatric interview, may be of concern. Similarly, despite the standardised and validated nature of the test of academic achievement, it is plausible that children's performance may be impacted by the extent of their exposure to the test content as part of the school curriculum. Reporting bias will be addressed by performing validation of statistical models and examining the effect of potential confounding variables (including perinatal variables, patient age, gender and socioeconomic status) when constructing models in order to better elucidate the true relationship between brain structure and outcomes at early, TEA and 6 years CA.

## Ethics and regulatory aspects

### Ethics

Ethical approval has been obtained from the Human Research Ethics Committees at the Children's Health Queensland Hospital and Health Service (HREC/19/QCHQ/49800) and The University of Queensland (2019000426). Participation in this study is voluntary, written informed consent will be obtained from a parent or guardian and families may withdraw from the study at any time without explanation.

### Participant safety

There are no known health or safety risks related to any aspect of the study. Assessments will be carried out over 2 days to minimise burden and fatigue to the child and family. The MRI and EEG are currently approved by the relevant authorities for use in humans. They have been used clinically and in research in children for a long time with no report of harm or discomfort. The MRI and EEG are non-invasive modalities and involve no ionising radiation or injections. They will be done while the child is awake with no need for anaesthesia or sedation. Children will be screened before the EEG and any with a diagnosis of epilepsy, recent head injury or who take medications that alter background EEG (drowsiness) will be ineligible for an EEG.

Prior to the MRI, all children will undertake an MRI checklist to ensure it is safe for them to be imaged. Earphones will be placed over the child's ears to reduce the noise of the MRI and they will be able to watch a movie and listen through the headphones. Some children may experience mild claustrophobia, as with standard clinical scans. The research team and radiographers are trained to deal with these situations and the MRI procedure can be discontinued at any time. Children may withdraw from the MRI procedure at any time and remain in the rest of the study.

## Unexpected findings during examinations

### *Magnetic resonance imaging*

The structural sequences of the MRIs will be reviewed in the Medical Imaging Department at RBWH (JB) and issued with a brief report which is forwarded to the chief investigator (JG). In the unlikely event of an unexpected finding, the radiologist will specify whether further follow-up is required (which may include a diagnostic MRI) or if the finding is of no clinical significance. If deemed medically appropriate, the parent/guardian of the child will be notified and MRI scans shared with the child's treating clinician to review in the public health system in the usual manner. If the child has no treating clinician, a medical referral will be arranged to ensure appropriate medical follow-up and management. All MRI reports will be provided to the child's general medical practitioner.

### *Neurodevelopmental assessment*

All families will receive a written report including the most clinically useful and interpretable elements of the clinical assessments. Every report will be reviewed by chief investigators (JG and SB). Families can share this report with their medical team or provide to their general medical practitioner if onward referral is indicated.

### *Dissemination*

The results of the study will be published in conference abstracts and presentations, peer-reviewed articles in scientific journals and in newsletters and media releases distributed by the study team. At the conclusion of the study after the primary analyses, a summary flyer of the main outcomes of the study will be emailed and/or mailed to the families.

## DISCUSSION

The outcomes of this unique prospective longitudinal birth cohort will include: (i) a novel and comprehensive description of the early and TEA MRI, EEG and clinical correlates in the preterm infant and the predictive validity for neurodevelopmental outcome at 6 years CA and (ii) identify biomarkers that could become tools for evaluation of neuroprotective therapies and be used as prognostic biomarkers for neurodevelopmental impairments. Earlier identification of infants at risk of adverse outcomes has the potential to provide opportunities for the evaluation of treatments while infants are still in the neonatal intensive care unit (ie, magnesium sulfate, cooling, caffeine and erythropoietin (EPO)).

The significance is that (i) parents and guardians will have more accurate prognostic information and counselling; (ii) clinical researchers will have tools to assist in the assessment and safety of neuroprotection, neurorestoration and neurorehabilitation interventions; (iii) infants at risk of neurodevelopmental delay/deficit, CP and psychiatric disorders will be detected earlier, leading to (iv) improved risk stratification for

earlier implementation of targeted interventions aimed at improving neurodevelopmental outcomes and (v) a reduction in neurodevelopmental disability and its high financial costs to individuals, families and society.

A limitation of this project is that care must be taken with interpretations based on the early and TEA MRI data, which are subject to motion artefacts and often have lower resolution than paediatric and adult scans. Our tailored MRI preprocessing strategy is critical to ensure optimal data quality before the models are constructed. Care must also be taken to ensure that models are not overfitted to these data, hence analyses on the sensitivity of the model based on the inputs and model overfitting will be investigated using bootstrap resampling. This validation will support the generalisability of the study results to other cohorts.

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