THE UNIVERSITY OF QUEENSLAND

AUSTRALIA

The relationship between brain structure and function of very preterm infants, and the ability to predict neurodevelopmental outcomes

Joanne Margaret George

BSc Physiotherapy (Hons)

A thesis submitted for the degree of Doctor of Philosophy at

The University of Queensland in 2017

Faculty of Medicine

Abstract

Background: Prediction of motor outcomes and cerebral palsy (CP) in infants born very preterm is needed to identify infants that may benefit from targeted intervention. Brain MRI at term equivalent age in very preterm infants has demonstrated predictive value for CP and adverse motor outcomes. Accuracy is further enhanced when MRI is combined with clinical measures of motor or neurological function. There is a need to determine if MRI and clinical biomarkers earlier than term equivalent age can determine motor outcomes. This would create a new window for possible intervention at a time of greater neuroplasticity and brain development.

Aims: To: i) systematically review the evidence for early MRI to identify infants with adverse motor outcomes or CP; ii) validate a structural MRI scoring system of brain injury and growth impairment (Kidokoro *et al, 2013*) at 29-35 weeks postmenstrual age (PMA, 'Early MRI'); iii) elucidate motor, neurological and neurobehavioural associations with structural MRI scores at Early and Term (40-42 weeks PMA) MRI; and iv) examine relationships between Early and Term MRI diffusion measures in the corpus callosum, posterior limb of the internal capsule (PLIC) and cerebral peduncle, and 12 month motor outcomes.

Research design and methodology: A prospective cohort study was conducted of infants born <31 weeks gestational age. Infants underwent Early and Term 3T MRI without sedation utilising an MRI compatible incubator. Concurrent clinical assessment performed within a week of each MRI consisted of the General Movements assessment (GMs), Hammersmith Neonatal Neurological Examination and the NICU Neonatal Neurobehavioural Scale. The Premie-Neuro was performed following Early MRI and the Test of Infant Motor Performance (TIMP) and a visual assessment at Term. Follow up at 3 months corrected age consisted of the GMs, TIMP and a visual assessment. At 12 months corrected age, infants were evaluated by a paediatrician for evidence of CP using a structured neurological examination. The Bayley Scales of Infant and Toddler Development 3rd edition (Bayley III), Alberta Infant Motor Scale (AIMS) and Neurosensory Motor Developmental Assessment (NSMDA) were conducted.

The structural MRI scoring system generated white matter (WM), cortical gray matter (GM), deep GM, cerebellar and global scores. Inter- and intrarater reliability and agreement of each of the MRI subscale scores and the overall global score were evaluated. The relationship between MRI scores and 12 month motor and cognitive outcomes were examined. Associations with concurrent clinical measures were assessed. Fractional anisotropy (FA) and mean diffusivity (MD) were calculated for the corpus callosum, PLIC and cerebral peduncle, where regions were extracted using registration

to the Johns Hopkins University neonatal Atlas. Relationships with 12 month motor and neurological outcomes were examined.

Results: The systematic review and meta-analyses revealed that Early structural MRI had satisfactory sensitivity and specificity to determine adverse motor outcomes and CP but that evidence for diffusion MRI was still emerging. Reproducibility was demonstrated for the structural MRI scoring system with good reliability and agreement for the overall score and all subscales except for cortical GM. Early MRI global, WM, and deep GM scores were negatively associated with Bayley III motor (regression coefficient for global score ß=-1.31; 95%CI=-2.39, -0.23; p=0.02; r^2=0.07), cognitive (ß=-1.52; 95%CI=-2.39, -0.65; p<0.01; r^2=0.16) and NSMDA outcomes (ß=-1.73; 95%CI=-3.19, -0.28; p=0.02; r^2=0.09). Early MRI cerebellar scores were negatively associated with the NSMDA (ß = -5.99; 95% CI, -11.82, -0.16; p = 0.04; r^2=0.08). Associations were reconfirmed at Term MRI and cerebellar scores were also associated with Bayley III and NSMDA outcomes.

Structural MRI scores were associated with concurrent motor, neurological and neurobehavioral function at Early and Term MRI. At Early MRI, cerebellar scores demonstrated the strongest associations with clinical measures, displaying associations with neurological and motor items but not neurobehavioural items. At Term MRI, the strongest associations were with motor performance measured with the TIMP. White matter abnormality scores were related to motor and neurological performance at Term but not at Early MRI.

Early MRI FA and MD in the defined regions were not associated with motor or neurological outcomes. Term MRI FA was not associated with outcomes. Term MRI MD in the left corpus callosum was associated with neurological outcome. Term MRI MD in the right cerebral peduncle was associated with motor outcome on the AIMS and NSMDA.

Conclusions: Early structural MRI is clinically accessible; however, limited reporting of diagnostic accuracy in Early MRI studies currently restricts clinical utility and translation to clinical practice. The Early structural MRI scoring system is valid for use between 29 and 35 weeks PMA, and is the first to incorporate qualitative evaluation of brain injury and evidence of growth impairment; as well as assessment of deep GM and the cerebellum. Early MRI diffusion measures of FA and MD in the corpus callosum, PLIC and cerebral peduncle were not associated with motor or neurological outcomes. The use of automatic segmentation methods to derive brain regions of interest resulted in exclusion of infants with significant structural brain lesions, possibly limiting the ability to find associations between the diffusion measures and 12 month motor outcomes.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

I acknowledge that an electronic copy of my thesis must be lodged with the University Library and, subject to the policy and procedures of The University of Queensland, the thesis be made available for research and study in accordance with the Copyright Act 1968 unless a period of embargo has been approved by the Dean of the Graduate School.

I acknowledge that copyright of all material contained in my thesis resides with the copyright holder(s) of that material. Where appropriate I have obtained copyright permission from the copyright holder to reproduce material in this thesis.

Publications during candidature

Published papers

1. George JM, Boyd RN, Colditz PB, Rose SE, Pannek K, Fripp J, Lingwood BE, Lai MM, Kong AH, Ware RS, Coulthard A, Finn CM, Bandaranayake SE. PPREMO: a prospective cohort study of preterm infant brain structure and function to predict neurodevelopmental outcome. *BMC Pediatr* 2015; **15**: 123.
2. George JM, Fiori S, Fripp J, Pannek K, Bursle J, Moldrich RX, Guzzetta A, Coulthard A, Ware RS, Rose SE, Colditz PB, Boyd RN. Validation of an MRI Brain Injury and Growth Scoring System in Very Preterm Infants Scanned at 29- to 35-Week Postmenstrual Age. *AJNR Am J Neuroradiol* 2017: May 18. doi: 0.3174/ajnr.A5191 [Epub ahead of print].

Published Conference Abstracts

1. Pannek K, George J, Colditz P, Boyd R, Rose S. Advanced diffusion weighted magnetic resonance imaging of the extremely preterm infant's brain. Australasian Academy of Cerebral Palsy and Developmental Medicine Conference (AusACPDM); Hunter Valley, Australia: *Developmental Medicine and Child Neurology* 2014; **56**(s2):63. doi: 10.1111/dmcn.12367
2. George J, Fripp J, Pannek K, Fiori S, Guzzetta A, Ware R, Rose S, Colditz P, Boyd R. Very early brain structure and neurological function detects brain injury in preterm infants at 30 weeks and 40 weeks postmenstrual age. 27th European Academy of Childhood Disability Conference (EACD); Copenhagen, Denmark: *Developmental Medicine and Child Neurology* 2015; **57**(s4):33. doi: 10.1111/dmcn.12780_20
3. George J, Fripp J, Pannek K, Fiori S, Guzzetta A, Ware R, Rose S, Colditz P, Boyd R. Very early brain structure and neurological function detects brain injury in preterm infants at 30 weeks and 40 weeks postmenstrual age. The 69th Annual Meeting of the American Academy for Cerebral Palsy and Developmental Medicine (AACPDM); Austin, Texas, USA: *Developmental Medicine and Child Neurology* 2015; **57**(s5):51-2. doi: 10.1111/dmcn.82_12887
4. George J, Fripp J, Shen K, Mitra J, Pannek K, Chan A, Ware R, Rose S, Colditz P, Boyd R. Relationship between early brain structure on MRI, white matter integrity (diffusion MRI) and neurological function at 30 weeks post menstrual age in infants born very preterm. 27th European Academy of Childhood Disability Conference (EACD); Copenhagen, Denmark: *Developmental Medicine and Child Neurology* 2015; **57**(s4):8-9. doi: 10.1111/dmcn.12778_10
5. George J, Fripp J, Shen K, Mitra J, Pannek K, Chan A, Ware R, Rose S, Colditz P, Boyd R. Relationship between early brain structure on MRI, white matter integrity (diffusion MRI) and

neurological function at 30 weeks post menstrual age in infants born very preterm. The 69th Annual Meeting of the American Academy for Cerebral Palsy and Developmental Medicine (AACPDM); Austin, Texas, United States: *Developmental Medicine and Child Neurology* 2015; **57**(s5):88-9. doi: 10.1111/dmcn23_12886

6. George J, Fripp J, Pannek K, Fiori S, Guzzetta A, Ware R, Rose S, Colditz P, Boyd R. MRI and clinical measures at 30 weeks postmenstrual age – early biomarkers for motor outcome at term equivalent age in infants born very preterm. Perinatal Society of Australia and New Zealand Conference (PSANZ); Townsville, Australia: *Journal of Paediatrics and Child Health* 2016; **52**:11. doi: 10.1111/jpc.13194

7. George J, Fripp J, Pannek K, Fiori S, Ware R, Rose S, Colditz P, Boyd R. Earlier detection of brain structure and functional biomarkers in in preterm infants at 30 and 40 weeks postmenstrual age. Australasian Academy of Cerebral Palsy and Developmental Medicine (AusACPDM); Adelaide, Australia: *Developmental Medicine and Child Neurology* 2016; **58**(s3):6-58. doi: 10.1111/dmcn.13069

8. George J, Fripp J, Shen K, Pannek K, Chan A, Ware R, Rose S, Colditz P, Boyd R. Relationship between white matter integrity at 3T MRI and neurological function in preterm infants at 30 weeks postmenstrual age. Australasian Academy of Cerebral Palsy and Developmental Medicine (AusACPDM); Adelaide, Australia: *Developmental Medicine and Child Neurology* 2016; **58**(s3):6-58. doi: 10.1111/dmcn.13069

9. George J, Fripp J, Shen K, Pannek K, Chan A, Ware R, Rose S, Colditz P, Boyd R. White matter microstructure at 30 weeks postmenstrual age is associated with motor outcome at 40 weeks postmenstrual age in infants born very preterm. Perinatal Society of Australia and New Zealand Conference (PSANZ); Townsville, Australia: *Journal of Paediatrics and Child Health* 2016; **52**:12-3. doi: 10.1111/jpc.13194

10. George J, Shen K, Fripp J, Pannek K, Ware R, Boyd R, Colditz P, Rose S. Cortical plate microstructure at 30 weeks postmenstrual age is associated with neurological function at term equivalent age in very preterm neonates: A diffusion MRI study. Perinatal Society of Australia and New Zealand Conference (PSANZ); Townsville, Australia: *Journal of Paediatrics and Child Health* 2016; **52**:8. doi: 10.1111/jpc.13194

11. Pannek K, George J, Colditz P, Boyd R, Rose S. Radial coherence of cortical grey matter fibres of very preterm infants undergoing advanced diffusion magnetic resonance imaging at 30 and 40 weeks postmenstrual age. Perinatal Society of Australia and New Zealand (PSANZ); Townsville, Australia: *Journal of Paediatrics and Child Health* 2016; **52**:12. doi: 10.1111/jpc.13194

12. George J, Fiori S, Fripp J, Pannek K, Guzzetta A, Coulthard A, Ware R, Rose S, Colditz P, Boyd R. New structural MRI scoring system at 29-35 weeks postmenstrual age is associated with motor outcomes at 12 months corrected age in infants born very preterm. 29th European Academy of Childhood Disability Conference (EACD); Amsterdam, The Netherlands: *Developmental Medicine and Child Neurology* 2017; **59**:21. doi: 10.1111/dmcn.13455

13. George J, Fiori S, Fripp J, Pannek K, Guzzetta A, Coulthard A, Ware R, Rose S, Colditz P, Boyd R. Brain structure at 29–35 weeks postmenstrual age is related to cognitive outcomes at 12 months corrected age in infants born very preterm. 29th European Academy of Childhood Disability Conference (EACD); Amsterdam, The Netherlands: *Developmental Medicine and Child Neurology* 2017; **59**:64. doi: 10.1111/dmcn.13456

Conference Paper Presentations

1. George J, Fripp J, Pannek K, Fiori S, Guzzetta A, Ware R, Rose S, Colditz P, Boyd R. Very early brain structure and neurological function detects brain injury in preterm infants at 30 weeks and 40 weeks postmenstrual age. Australian Physiotherapy Association (APA); Gold Coast, QLD, Australia; 2015.

2. George J, Fripp J, Pannek K, Fiori S, Guzzetta A, Ware R, Rose S, Colditz P, Boyd R. Very early brain structure and neurological function detects brain injury in preterm infants at 30 weeks and 40 weeks postmenstrual age. The 69th Annual Meeting of the American Academy for Cerebral Palsy and Developmental Medicine (AACPDM); Austin, Texas, USA: *Developmental Medicine and Child Neurology* 2015; **57**(s5):51-2. doi: 10.1111/dmcn.82_12887

3. George J, Fripp J, Shen K, Mitra J, Pannek K, Chan A, Ware R, Rose S, Colditz P, Boyd R. Relationship between early brain structure on MRI, white matter integrity (diffusion MRI) and neurological function at 30 weeks post menstrual age in infants born very preterm. 27th European Academy of Childhood Disability Conference (EACD); Copenhagen, Denmark: *Developmental Medicine and Child Neurology* 2015; **57**(s4):8-9. doi: 10.1111/dmcn.12778_10

4. George J, Fripp J, Shen K, Mitra J, Pannek K, Chan A, Ware R, Rose S, Colditz P, Boyd R. Relationship between early brain structure on MRI, white matter integrity (diffusion MRI) and neurological function at 30 weeks postmenstrual age in infants born very preterm. Australian Physiotherapy Association (APA); Gold Coast, QLD, Australia; 2015.

5. George J, Fripp J, Pannek K, Fiori S, Ware R, Rose S, Colditz P, Boyd R. Earlier detection of brain structure and functional biomarkers in in preterm infants at 30 and 40 weeks postmenstrual age. Australasian Academy of Cerebral Palsy and Developmental Medicine (AusACPDM); Adelaide, Australia: *Developmental Medicine and Child Neurology* 2016; **58**(s3):6-58. doi: 10.1111/dmcn.13069

6. George J, Fripp J, Shen K, Pannek K, Chan A, Ware R, Rose S, Colditz P, Boyd R. Relationship between white matter integrity at 3T MRI and neurological function in preterm infants at 30 weeks postmenstrual age. Australasian Academy of Cerebral Palsy and Developmental Medicine (AusACPDM); Adelaide, Australia: *Developmental Medicine and Child Neurology* 2016; **58**(s3):6-58. doi: 10.1111/dmcn.13069

7. George J, Shen K, Fripp J, Pannek K, Ware R, Boyd R, Colditz P, Rose S. Cortical plate microstructure at 30 weeks postmenstrual age is associated with neurological function at term equivalent age in very preterm neonates: A diffusion MRI study. Perinatal Society of Australia and New Zealand Conference (PSANZ); Townsville, Australia: *Journal of Paediatrics and Child Health* 2016; **52**:8. doi: 10.1111/jpc.13194

8. George J, Fiori S, Fripp J, Pannek K, Guzzetta A, Coulthard A, Ware R, Rose S, Colditz P, Boyd R. New structural MRI scoring system at 29-35 weeks postmenstrual age is associated with motor outcomes at 12 months corrected age in infants born very preterm. 29th European Academy of Childhood Disability Conference (EACD); Amsterdam, The Netherlands: *Developmental Medicine and Child Neurology* 2017; **59**:21. doi: 10.1111/dmcn.13455

Conference Poster Presentations

1. Pannek K, George J, Colditz P, Boyd R, Rose S. Early high b-value diffusion MRI of the preterm infant brain highlights radial organisation of the developing cortex. International Society for Magnetic Resonance in Medicine (ISMRM); Milan, Italy; 2014.

2. Pannek K, George J, Colditz P, Boyd R, Rose S. Advanced diffusion weighted magnetic resonance imaging of the extremely preterm infant's brain. Australasian Academy of Cerebral Palsy and Developmental Medicine Conference (AusACPDM); Hunter Valley, Australia: *Developmental Medicine and Child Neurology* 2014; **56**(s2):63. doi: 10.1111/dmcn.12367

3. George J, Fripp J, Pannek K, Fiori S, Guzzetta A, Ware R, Rose S, Colditz P, Boyd R. Very early brain structure and neurological function detects brain injury in preterm infants at 30 weeks and 40 weeks postmenstrual age. 27th European Academy of Childhood Disability Conference (EACD); Copenhagen, Denmark: *Developmental Medicine and Child Neurology* 2015; **57**(s4):33. doi: 10.1111/dmcn.12780_20

4. George J, Fripp J, Shen K, Mitra J, Pannek K, Chan A, Ware R, Rose S, Colditz P, Boyd R. Relationship between early brain structure on MRI, white matter integrity (diffusion MRI) and neurological function at 30 weeks post menstrual age in infants born very preterm. The 69th Annual Meeting of the American Academy for Cerebral Palsy and Developmental Medicine (AACPDM); Austin, Texas, United States: *Developmental Medicine and Child Neurology* 2015; **57**(s5):88-9. doi: 10.1111/dmcn23_12886

5. Pannek K, George J, Boyd R, Colditz P, Rose S, Rueckert D. Combined slicewise and voxelwise outlier detection in diffusion MRI data in preterm born infants. Organization for Human Brain Mapping Conference (OHBM); Hawaii, USA; 2015.
6. George J, Fripp J, Pannek K, Fiori S, Guzzetta A, Ware R, Rose S, Colditz P, Boyd R. MRI and clinical measures at 30 weeks postmenstrual age – early biomarkers for motor outcome at term equivalent age in infants born very preterm. Perinatal Society of Australia and New Zealand Conference (PSANZ); Townsville, Australia: *Journal of Paediatrics and Child Health* 2016; **52**:11. doi: 10.1111/jpc.13194
7. George J, Fripp J, Pannek K, Fiori S, Guzzetta A, Ware R, Rose S, Colditz P, Boyd R. Very early brain structure and neurological function detects brain injury in preterm infants at 30 weeks and 40 weeks postmenstrual age. 28th European Academy of Childhood Disability Conference (EACD); Stockholm, Sweden; 2016.
8. George J, Fripp J, Shen K, Pannek K, Chan A, Ware R, Rose S, Colditz P, Boyd R. White matter microstructure at 30 weeks postmenstrual age is associated with motor outcome at 40 weeks postmenstrual age in infants born very preterm. Perinatal Society of Australia and New Zealand Conference (PSANZ); Townsville, Australia: *Journal of Paediatrics and Child Health* 2016; **52**:12-3. doi: 10.1111/jpc.13194
9. Kong A, Finnigan S, Ware R, Finn C, George J, Boyd R, Colditz P. Early EEG markers may inform prognostication of cognitive outcomes in very preterm infants. 14th International Child Neurology Congress; Amsterdam, The Netherlands; 2016.
10. Kong A, Finnigan S, Ware R, George J, Boyd R, Colditz P. Brain activity assessed using EEG predicts visual function at term equivalent age in very preterm infants 25th annual RBWH Healthcare Symposium; Brisbane, Australia; 2016.
11. McKeague S, George J, Ware R, Colditz P, Boyd R. Inter-rater reproducibility of the Hammersmith Neonatal Neurological Examination in very preterm infants at 30 and 40 weeks postmenstrual age. European Academy of Childhood Disability Conference (EACD); Stockholm, Sweden; 2016.
12. Pannek K, George J, Colditz P, Boyd R, Rose S. Radial coherence of cortical grey matter fibres of very preterm infants undergoing advanced diffusion magnetic resonance imaging at 30 and 40 weeks postmenstrual age. Perinatal Society of Australia and New Zealand (PSANZ); Townsville, Australia: *Journal of Paediatrics and Child Health* 2016; **52**:12. doi: 10.1111/jpc.13194
13. George J, Fiori S, Fripp J, Pannek K, Guzzetta A, Coulthard A, Ware R, Rose S, Colditz P, Boyd R. Brain structure at 29–35 weeks postmenstrual age is related to cognitive outcomes at 12 months corrected age in infants born very preterm. 29th European Academy of Childhood

Disability Conference (EACD); Amsterdam, The Netherlands: *Developmental Medicine and Child Neurology* 2017; **59**:64. doi: 10.1111/dmcn.13456

14. Pannek K, Fripp J, George J, Boyd R, Colditz P, Rose S. Automatic detection of volumes affected by subvolume motion in diffusion weighted imaging. International Symposium on Biomedical Imaging (ISBI); Melbourne, Australia; 2017.

15. Pannek K, Fripp J, George J, Boyd R, Colditz P, Rose S. Automatic detection of volumes affected by subvolume movement. International Society for Magnetic Resonance in Medicine (ISMRM); Honolulu, USA; 2017.

16. Shen K, Fripp J, Pannek K, George J, Colditz P, Boyd R, Rose S. A spatio-temporal atlas of neonatal diffusion MRI based on kernel ridge regression International Symposium on Biomedical Imaging (ISBI); Melbourne, Australia; 2017.

Conference Workshop/Symposium Presentations

1. Boyd R, Guzzetta A, George J, Whittingham K. Working with infants with CP: new research and future possibilities. Australasian Academy of Cerebral Palsy and Developmental Medicine (AusACPDM); Hunter Valley, Australia; 2014.

2. George J, Boyd R, Colditz P, Rose S. PPREMO – Prediction of PREterm Motor Outcomes. The relationship between brain structure and function of very preterm infants, and the ability to predict neurodevelopmental outcomes. Grantley Stable Neonatal Unit Conference (GSNU); Brisbane, Australia; 2014.

3. Boyd R, Fiori S, Guzzetta A, George J, Pannek K. PPREMO Toolbox: Very Early Brain Structure and Function to predict Motor and Brain outcomes in infants born preterm. 29th European Academy of Childhood Disability Conference (EACD); Amsterdam, The Netherlands; 2017.

Publications included in this thesis

1. George JM, Boyd RN, Colditz PB, Rose SE, Pannek K, Fripp J, Lingwood BE, Lai MM, Kong AH, Ware RS, Coulthard A, Finn CM, Bandaranayake SE. PPREMO: a prospective cohort study of preterm infant brain structure and function to predict neurodevelopmental outcome. *BMC Pediatr* 2015; **15**: 123.

Incorporated as Chapter 3

Contributor	**Statement of contribution**
Joanne George (Candidate)	Designed the study (40%); Ethics applications and reporting (100%); Statistical analysis plan (75%); Wrote manuscript (80%)
Roslyn Boyd	Designed the study (15%); Critical review of manuscript (45%)
Paul Colditz	Designed the study (10%); Wrote manuscript - EEG component (5%); Critical review of manuscript (15%)
Stephen Rose	Designed the study (10%); Critical review of manuscript (10%)
Kerstin Pannek	Designed the study (10%); Wrote manuscript - MRI component (10%); Critical review of manuscript (15%)
Jurgen Fripp	Designed the study (2.5%); Critical review of manuscript (15%)
Barbara Lingwood	Wrote manuscript - Nutrition component (2.5%)
Melissa Lai	Designed the study (2.5%)
Annice Kong	Designed the study (2.5%); Wrote manuscript - EEG component (2.5%)
Robert Ware	Statistical analysis plan (25%); Critical review of manuscript (10%)
Alan Coulthard	Designed the study (2.5%)
Christine Finn	Designed the study (2.5%)
Sasaka Bandaranayake	Designed the study (2.5%)

2. George JM, Fiori S, Fripp J, Pannek K, Bursle J, Moldrich RX, Guzzetta A, Coulthard A, Ware RS, Rose SE, Colditz PB, Boyd RN. Validation of an MRI Brain Injury and Growth Scoring System in Very Preterm Infants Scanned at 29- to 35-Week Postmenstrual Age. *AJNR Am J Neuroradiol* 2017: May 18. doi: 0.3174/ajnr.A5191 [Epub ahead of print].
Incorporated as Chapter 4.

Contributor	**Statement of contribution**
Joanne George (Candidate)	Study design (45%), statistical analysis (95%); interpretation of results (75%), wrote the paper (100%).
Simona Fiori	Study design (5%), scored all MRI's (100%), and edited paper (5%)
Jurgen Fripp	MRI data acquisition (50%), interpretation of results (5%), edited paper (5%)
Kerstin Pannek	Study design (10%), MRI data acquisition (50%), interpretation of results (5%), and edited paper (5%)
Jane Bursle	Second blinded scorer of MRI scans for reproducibility analyses (100%).
Randal Moldrich	Statistical analysis of data in Online tables 5 and 6 (5%).
Andrea Guzzetta	Study design (5%)
Alan Coulthard	Study design (5%); review and sanction of MRI scoring methodology and data (100%), edited paper (5%)
Robert Ware	Review and sanction of all statistical methodology, analysis and results (100%), edited paper (10%)
Stephen Rose	Study design (10%), edited paper (5%)
Paul Colditz	Study design (10%), interpretation of results (5%), edited paper (10%)
Roslyn Boyd	Study design (10%), interpretation of results (10%), edited paper (45%)

Contributions by others to the thesis

Professors Roslyn Boyd, Paul Colditz and Stephen Rose were responsible for study conception, procurement of initial funding for the study and formed the supervisory team. I designed the study protocol, completed ethics and governance applications and approvals, implemented, co-ordinated and managed the study under the guidance of the supervisory team. Roslyn Boyd, Paul Colditz, Stephen Rose and I secured funding for the project. Andrea Guzzetta assisted with study design.

Research nurses Kellie McGrory, Donna Hovey and Kylie Smart conducted recruitment. Research nurses collected perinatal and demographic data of study participants. I conducted clinical assessments and data collection at Early and Term assessment time points. Paul Colditz assisted with clinical data collection at the Early time point. Chris Finn, Kym Morris and I conducted the 3 month follow up assessments. Chris Finn and Kym Morris performed 12 month follow up assessments.

Kerstin Pannek and Stephen Rose designed the MRI acquisition protocol for the study. Kerstin Pannek or Jurgen Fripp attended all MRIs to supervise MRI data collection and quality control. Kerstin Pannek, Jurgen Fripp and Kaikai Shen performed all MRI data quality control, pre-processing and analysis of diffusion MRI data. Simona Fiori scored the structural MR images. Jane Bursle was the second rater for the reproducibility analyses of the structural MRI scoring system developed in this thesis. Randal Moldrich performed statistical analyses for the reproducibility study.

Professor Robert Ware, a biostatistician, supervised all aspects of statistical analysis. Alan Coulthard assisted with study design, in particular, the practical implementation of the MRI protocol in the Radiology department of the Royal Brisbane and Women's Hospital. Lars Eriksson, a librarian at The University of Queensland, assisted with development of search strategies for the systematic review in chapter 2. Chris Finn and Sasaka Bandaranayake assisted with designing the 12 month follow up neurological assessment. Michael David provided some additional statistical supervision.

Statement of parts of the thesis submitted to qualify for the award of another degree

None

Acknowledgements

This PhD has been a challenging and rewarding journey. I have been supported throughout by a remarkable, dynamic and wise supervisory team, a committed and skilled research group, and a magnificent network of family and friends.

Professor Roslyn Boyd is changing the landscape of preterm infant research in Queensland and Australia. I would like to thank her for her tireless time and energy she has invested in me as a PhD student, and in the PPREMO project. I would also like to thank her for teaching me so much in terms of research, academic writing, clinical study design, implementation and management. I really benefitted from having such a skilled and experienced primary supervisor.

I would like to thank Professor Paul Colditz for his clinical and academic mentoring. I enjoyed learning from him in the neonatal intensive care unit environment, and appreciated his skill in keeping a clear, clinical perspective in the research and academic setting. Professor Colditz had a respectful way of acknowledging where I was at in terms of my learning or understanding of a topic or situation. He had a skilful way of guiding and teaching me from there which I valued greatly. I would like to acknowledge Professor Stephen Rose for his supervision of the MRI elements of my PhD study and the PPREMO project. This project would not have been possible without his specialist and expert guidance in regards to MRI and I greatly appreciate the time and expertise he has contributed to my PhD. Professor Rob Ware supervised all aspects of statistical methodology and interpretation and presentation of results in this thesis. I thank him for his patience and instruction, and credit him with the gains in statistical understanding and ability I have made through this postgraduate study.

Kerstin Pannek contributed significantly to the design and implementation of the PPREMO project. Through the trials and tribulations of this challenging and exciting project, she has become a friend and a greatly respected colleague. Together with Jurgen Fripp and Kaikai Shen, they have been responsible for all MRI data analysis for the PPREMO study, no small task. All three have taught me a significant amount about MRI. In addition, I thank Jurgen Fripp for all he has taught me about statistics and interpretation of research results. I would like to thank Simona Fiori for scoring all the structural MR images and contributing a wealth of knowledge in regards to structural MRI. I would also like to thank Andrea Guzzetta for his consultation at key stages of the PPREMO project and in relation to papers which contribute to this thesis.

I would like to thank and acknowledge the exceptional team of clinical staff who supported the PPREMO project. Retention of 87% of the study cohort to 12 months corrected age is a testament to the sensitive and empathetic way they connected with our study participants and families, as well as their organizational skills and commitment. Their clinical skills and expertise guarantee that the data we have collected is of the highest quality and ensures confidence for all authors working with the PPREMO study data.

The University of Queensland, Queensland Government and Queensland Cerebral Palsy and Rehabilitation Research Centre (QCPRRC) supported me financially with research scholarships, without which I would not have been able to conduct this PhD full time. I am grateful for the wide range of resources provided to me through the university which supported my learning. I would like to thank the staff and students of QCPRRC for their assistance and support throughout this journey. Special thanks to Noel for the IT and graphics assistance and teaching.

My appreciation for my parents grows more and more with time as my insight into the significant blessings of my family and early education deepens. Their emotional intelligence and mentorship helped me keep a clear perspective and focus on the long view. My husband, Nick, deserves the greatest thanks of all. He is my champion. His gracious financial backing of this venture, unwavering support and sense of humour kept me going from the start and right up to this finish line. To my magnificent friends and family, you each played pivotal roles in getting me to this point, thank you. To my darling Jack, thank you for being so patient while mum has been working so hard – I can't wait to go camping with you again and do normal, fun stuff on the weekends! To dear Evelyn, you are a blessing to our little family.

None of this work would have been possible without the participation and support from families of preterm and term infants in this study. To them I owe the greatest debt of gratitude. Ultimately, it was parents of very preterm infants with adverse outcomes who inspired me to search for more evidence to support what we do as clinicians, and to find ways to improve outcomes for very preterm infants and their families. This work is dedicated to them.

Keywords

preterm, neurological, neuromotor, neurobehaviour, neurodevelopment, magnetic resonance imaging, prediction, outcomes, cerebral palsy, motor delay

Australian and New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code: 110317, Physiotherapy, 40%
ANZSRC code: 110903, Central Nervous System (or 110999 Neurosciences not elsewhere classified), 30%
ANZSRC code: 111499, Paediatrics and Reproductive medicine not elsewhere classified, 30%

Fields of Research (FoR) Classification

FoR code: 1103, Clinical Sciences, 40%
FoR code: 1109, Neurosciences, 30%
FoR code: 1114, Paediatrics and Reproductive medicine, 30%

Table of contents

List of Tables and Figures

Tables

Figures

List of abbreviations used in the thesis

AD	Axial Diffusivity
AIMS	Alberta Infant Motor Scale
ASL	Arterial Spin Labelling
Bayley III	Bayley Scales of Infant and Toddler Development, 3rd Edition
CA	Corrected age
CBF	Cerebral blood flow
CI	Confidence interval
CP	Cerebral palsy
CUS	Cranial Ultrasound
DTI	Diffusion Tensor Imaging
DWI	Diffusion Weighted Imaging
EEG	Electroencephalography
FA	Fractional Anisotropy
FOD	Fibre orientation distribution
GA	Gestational age
GMs	General Movements Assessment
HARDI	High Angular Resolution Diffusion Weighted Imaging
HNNE	Hammersmith Neonatal Neurological Examination
IVH	Intraventricular haemorrhage
MD	Mean Diffusivity
MND	Minor neurological deficit
MR	Magnetic resonance
MRI	Magnetic resonance imaging
NNNS	NICU Neonatal Neurobehavioural Scale
NODDI	Neurite orientation dispersion and density imaging
NSMDA	Neuro Sensory Motor Development Assessment
PMA	Postmenstrual age
PVL	Periventricular leukomalacia
RD	Radial Diffusivity
TEA	Term equivalent age
TIMP	Test of Infant Motor Performance

Chapter 1: Introduction, thesis outline and aims

1.1 Introduction

Preterm infants face a broad and diverse range of neurodevelopmental outcomes including cognitive, behavioural and motor deficits and cerebral palsy (CP) [1-5]. Identifying those at risk of adverse outcomes enables targeted interventions to be initiated and family supports to be instituted. Earlier identification of potential adverse outcomes ensures families receive adequate support and enables limited healthcare funds to be appropriately utilised.

Brain MRI in recent years has improved the ability to predict motor outcomes and determine infants at high risk of CP. A substantial body of evidence now exists for brain MRI at term-equivalent age (TEA) in infants born very preterm [6-8]. Structural MRI provides qualitative information of white and gray matter injuries and brain macrostructure[9-11]. Diffusion MRI and advanced diffusion acquisition and analysis techniques provide detailed information on brain microstructural development and maturation. Relationships with clinical biomarkers have been reported at TEA and predictive validity for later neurodevelopmental outcomes has been established [12-20].

The principal rationale for this thesis was to determine if it was possible to identify infants at risk of adverse motor outcomes and CP earlier than TEA using earlier MRI and clinical biomarkers. If achieved, a new window for interventions would be available at a time of rapid brain development and plasticity. Additionally, with the increasing propensity for very preterm infants being discharged prior to TEA, an earlier MRI assessment would allow for MRI prior to discharge from the neonatal intensive care unit. This would facilitate follow up and reduce the risk of loss to follow up, ensuring more infants undergo MRI rather than requiring families to return for assessment at TEA.

The most relevant study design to achieve this was a prospective cohort study. Structural and diffusion MRI were acquired at 30-32 weeks postmenstrual age (PMA, 'Early MRI'), and 40-42 weeks PMA ('Term MRI'). Concurrent clinical measures of motor, neurological and neurobehavioural function were obtained. Follow up at 12 months corrected age (CA) using validated tools and a neurological assessment was conducted to determine motor outcomes and the risk of CP. It was acknowledged that 12 months corrected age was too early for a reliable diagnosis of CP. Added to the trend of a decreasing incidence of CP in very preterm infants[21-23], it was recognised that prevalence of CP was likely to be low in our contemporaneous cohort. For these reasons, the focus of this thesis will be motor outcomes measured using validated tools.

Aims of this thesis were to: i) systematically review the literature to evaluate the current evidence for Early MRI to determine adverse motor outcomes and CP; ii) validate a structural MRI scoring system of brain injury and growth impairment (Kidokoro *et al, 2013*) for use at 29-35 weeks postmenstrual age (PMA, 'Early MRI') by demonstrating reproducibility and associations with 12 month neurodevelopmental outcomes; iii) elucidate motor, neurological and neurobehavioural correlates for structural MRI scores at Early and Term (40-42 weeks PMA) MRI; and iv) examine the relationships between Early and Term MRI diffusion measures in the corpus callosum, posterior limb of the internal capsule (PLIC) and cerebral peduncle, and 12 month motor outcomes. In order to address these aims, this thesis presents the following:

- A systematic review of the literature which examined the relationships between Early MRI and motor outcomes in infants born very preterm in Chapter 2.
- A detailed study protocol which described the methodology for the broader prospective cohort study within which this thesis is embedded, in Chapter 3.
- Validation of an MRI brain injury and growth scoring system for Early MRI structural images. This was achieved by demonstrating associations with later motor and cognitive outcomes at 12 months CA and is presented in Chapter 4.
- Results of the relationships between the Early structural MRI scores and concurrent neuromotor, neurological and neurobehavioral function in Chapter 5.
- Results of the relationships between Early and Term MRI diffusion measures of brain microstructure and 12-month motor outcome in Chapter 6.
- A grand discussion which synthesises the study findings, highlights study strengths and limitations and details clinical and research implications, in Chapter 7.

1.2 Aims

Aim 1

To examine the diagnostic accuracy of early MRI (<36 weeks PMA) to determine adverse motor outcomes or a confirmed diagnosis of CP, at or beyond one year CA in infants born preterm.

Aim 2

To validate a structural MRI scoring system previously developed for very preterm infants at TEA in a cohort of infants born <31 weeks PMA with MRI between 29 and 35 weeks PMA. The study aimed to establish predictive validity for motor and cognitive outcomes at 12 months CA. Secondary aims were to examine inter- and intrarater reproducibility and to examine relationships between global brain abnormality categories and known perinatal risk factors.

Aim 3

To examine the structure-function relationships between structural MRI brain abnormality scores and concurrent clinical measures of neuromotor, neurological and neurobehavioral performance at 30-32 weeks PMA (Early MRI) and again at 40-42 weeks PMA (Term MRI). A secondary aim was to evaluate which clinical measures demonstrated the strongest association with a) Early MRI and b) Term MRI.

Aim 4

To evaluate brain microstructure on Early and Term MRI in regions known to be involved in motor function, the corpus callosum, posterior limb of the internal capsule, and cerebral peduncle and then examine the association of these early microstructural measures with motor outcome at 12 months CA. A secondary aim was to compare preterm brain microstructure in the 3 defined regions at Term MRI with a term born reference sample imaged at approximately 40-42 weeks PMA.

1.3 Format of Thesis

This thesis consists of a sequence of papers published or submitted to peer-reviewed journals. Chapter 2 presents a systematic review of the literature which examined the associations between early MRI and later motor outcomes or cerebral palsy, and addressed the first aim of the thesis. Chapter 3 introduces the study protocol. The conclusion to Chapter 3 details the order in which this thesis was completed in parallel to progress of the prospective cohort study. It outlines in detail why different papers used different sample sizes as adequate thresholds were reached to address each specified aim. Chapter 4 presents the validation of an MRI scoring system for structural MR images at 29-35 weeks PMA. Chapter 5 then examines the cross-sectional relationships between the validated MRI scores and concurrent clinical measures of motor, neurological and neurobehavioural function. Chapter 6 evaluates brain microstructure using diffusion MRI, and in a similar process to the validation of structural MRI scoring, examines relationships with 12 month motor outcomes. The conclusion of Chapter six includes evaluation of the representativeness of the overall cohort, and comparisons between each sub-sample included in the separate papers and the overall recruited sample. The grand discussion in Chapter 7 synthesizes the findings in a detailed summary and conclusion, followed by study limitations, implications for clinical practise and recommendations for future research.

Chapter 2: Diagnostic accuracy of early MRI to determine motor outcomes in infants born preterm: a systematic review and meta-analysis

2.1 Introduction to Chapter 2

In order to review the current literature on early MRI in babies born preterm and the ability of MRI to determine motor outcome on validated tools, a systematic review and meta-analysis was undertaken.

2.2 Paper 1:

This manuscript has been submitted to the journal *Developmental Medicine and Child Neurology* and is currently under review (journal impact factor 3.615).

George JM, Pannek K, Rose SE, Ware RS, Colditz PB, Boyd RN. Diagnostic accuracy of early MRI to determine motor outcomes in infants born preterm: a systematic review and meta-analysis.

Diagnostic accuracy of early MRI to determine motor outcomes in infants born preterm: a systematic review and meta-analysis

George JM, Pannek K, Rose SE, Ware RS, Colditz PB, Boyd RN

Abstract

Aim To examine the diagnostic ability of early MRI (<36 weeks postmenstrual age) to detect later adverse motor outcomes or cerebral palsy (CP) in infants born preterm.

Method Studies of preterm infants with MRI <36 weeks postmenstrual age and quantitative motor data or a diagnosis of CP ≥one year corrected age were identified. Study details were extracted and meta-analyses performed where possible. Quality of included studies was evaluated with the QUADAS-2 tool.

Results Thirty articles met criteria of which five reported diagnostic accuracy and five reported data sufficient for calculation of diagnostic accuracy. Early structural MRI global scores detected a later diagnosis of CP with pooled sensitivity 100% (95% confidence interval CI 86-100%) and specificity 89% (95%CI 54-100%). Global structural MRI scores determined adverse motor outcomes with pooled sensitivity 89% (95%CI 44-100%) and specificity 96% (95%CI 85-100%). White matter scores determined adverse motor outcomes with pooled sensitivity 33% (95% CI 20-48%) and specificity 83% (95% CI 78-88%).

Interpretation Early structural MRI has reasonable sensitivity and specificity to determine adverse motor outcomes and CP in infants born preterm. Greater reporting of diagnostic accuracy in studies examining relationships with motor outcomes and CP is required to facilitate clinical utility of early MRI.

What this paper adds

- Early MRI has reasonable sensitivity and specificity to determine later adverse motor outcomes and CP in infants born preterm;
- Detection of infants who progressed to CP was stronger than motor outcomes;
- Global MRI scores discriminated between infants with normal and adverse motor outcomes more accurately than WM scores;
- Few studies report diagnostic accuracy of early MRI findings;
- Diagnostic accuracy is required to draw clinically meaningful conclusions from studies reporting associations between early MRI and motor outcomes/CP.

Cerebral palsy (CP) results from an early brain injury that in approximately 70 percent of cases occurs in the third trimester of pregnancy or around birth[1]. Preterm birth is the single greatest risk factor for CP with approximately 43% of infants diagnosed with CP born preterm[2]. The risk of CP increases with decreasing gestational age (GA) at birth, with approximately 5-10% of infants born <30 weeks GA developing CP[3, 4]. In infants born <30 weeks postmenstrual age (PMA) who do not develop CP, there is a significant risk for adverse motor outcomes which range from mild to severe motor impairment[5]. Early identification of infants at risk of adverse motor outcomes and CP is required to counsel families and refer them to early interventions.

Magnetic resonance imaging (MRI) at term equivalent age (TEA) has been shown to identify infants with CP and motor outcomes at or beyond two years corrected age (CA) in infants born very preterm [3, 6-8]. In a systematic review of tests to predict CP in high risk cohorts, MRI at TEA determined a later outcome of CP with a sensitivity of 86-100% and specificity of 89-97%[6, 7, 9]. Another systematic review of TEA MRI in preterm born infants reported a sensitivity of 77% and specificity of 79% to determine an outcome of CP, and sensitivity 72% and specificity 62% to determine motor outcome[10].

A recent systematic review of advanced neuroimaging at TEA summarized biomarkers associated with neurodevelopmental outcomes in preterm infants[11]. Biomarkers identified included tissue volumes and metrics of microstructural integrity and maturation based on diffusion MRI such as fractional anisotropy (FA) and/or mean, radial or axial diffusivity (MD, RD, AD)[11]. Brain regions with evidence in three or more studies included the corpus callosum, cerebellum, centrum semiovale, sensorimotor white matter (WM), subcortical nuclei and posterior limb of the internal capsule (PLIC)[11]. A number of non-systematic reviews have summarized structural and diffusion imaging at TEA and the associations with neurodevelopmental outcomes in infants born very preterm[12-14]. Structural MRI at TEA was strongly associated with neurodevelopmental outcomes, while evidence of advanced imaging biomarkers to determine neurodevelopmental outcomes was emerging[12-14].

To date, one systematic review has evaluated MRI earlier in the neonatal period (<36 weeks PMA)[15]. The authors concluded that TEA MRI afforded greater prognostic information than early MRI, and emphasized the importance of early MRI for research into early brain injury and development. Early MRI (before 36 weeks PMA) has become more widespread with the increasing availability of MR compatible incubators[16]. Further systematic evaluation of the literature to determine the ability of early MRI to accurately determine neurodevelopmental outcomes and CP is

warranted. The aim of this systematic review was to examine the diagnostic accuracy of early MRI (<36 weeks PMA) to determine adverse motor outcomes or a confirmed diagnosis of CP at or beyond one year CA in infants born preterm.

METHOD

Search Strategy

Databases searched were PubMed, EMBASE, Cochrane, CINAHL and Scopus from inception to 31 March 2017. Keywords (preterm OR infant, premature) AND (MRI OR magnetic resonance imaging OR MR OR magnetic resonance OR dti OR diffusion) AND (motor OR neuromotor OR Bayley OR AIMS OR NSMDA OR Griffith OR MABC OR cerebral palsy OR CP) were used and studies were limited to those published in English (Supplementary Material A).

Studies were eligible for inclusion if participants were born preterm (<36 weeks PMA) and the sample size was ≥10. The participant's MRI was performed at <36 weeks PMA with structural, diffusion, spectroscopic (MRS) and/or functional MRI (fMRI) sequences acquired and MRI analysis by a reproducible qualitative or quantitative method. Quantitative motor outcome data from validated tools and/or a confirmed diagnosis of CP at or beyond 12 months corrected age was the final eligibility criteria. Studies of normative samples of preterm infants, i.e. no evidence of brain injury and normal motor outcomes on standardised tests, were excluded. Studies were excluded if brain injuries were the result of acute/traumatic brain injury or congenital malformations.

Data extraction and analysis

Three reviewers (JG, KP, and RB) independently screened the titles and abstracts, then examined full text articles where required to determine eligibility. Disagreements were resolved through discussion. Demographic data extracted included: study design, sample size, GA at birth, birth weight, sex, and PMA at early MRI. The MRI details extracted were MRI field strength, acquisition type, analysis type and qualitative or quantitative MRI findings. Motor outcome data included: number of participants with follow up data, age at follow up, validated tool utilized, quantitative motor data, number of participants diagnosed with CP and detail of CP motor distribution and severity where available. Where participants were assessed at more than one time point >12 months CA, the data from the later assessment was utilized.

Diagnostic accuracy can be characterized using a number of possible measures. In this review, sensitivity and specificity were chosen *a priori* as the primary outcome measures as they are not affected by the prevalence of the underlying condition, and consequently data from heterogeneous

populations could be combined. Sensitivity and specificity of MRI abnormalities to determine adverse motor outcomes and/or a diagnosis of CP was extracted where reported, or calculated from raw data. Positive and negative predictive values were not reported as the prevalence of adverse motor outcomes and CP in a cohort affects the ability to predict outcomes from early MRI, which limits the external validity of results[17, 18]. Diagnostic statistics are presented as a point estimate and 95% confidence interval (95%CI). Quality of included studies was evaluated with the QUADAS-2 tool, which is comprised of four domains: patient selection, index test, reference standard, and flow and timing[19]. Each domain is assessed in terms of risk of bias, and the first three domains are also assessed in terms of concerns regarding applicability. Each domain is given one of three possible ratings: low risk, high risk, or unclear risk. Meta-analysis was conducted where possible using Stata version 14 (StataCorp, College Station, TX, USA).

RESULTS

Search results

The title and abstracts of 813 records and 114 full text articles were retrieved and reviewed to assess eligibility (On-line Figure 1). Twenty-five studies met the inclusion criteria and five further studies were identified by manual review of references of included papers[20-24]. Demographic and clinical data extracted from the 30 studies included in this review are presented in Table 1. The type of imaging modalities utilized in included studies are presented in Figure 1.

Characteristics of included studies

Of the 30 included studies, 16 distinct cohorts were identified and a further 14 studies reported data of different analyses of either the same or partially overlapping cohorts. The predominant study design was prospective cohort study. Sample sizes ranged from 23-193 participants, with all but two studies recruiting only infants born <36 weeks PMA. Excluding the studies with infants born ≥36 weeks PMA, birthweight ranged from 370-2965 grams. The proportion of males ranged from 38-76%. The PMA at early MRI ranged from 25.9-46.3 weeks. Thirteen studies of six distinct cohorts had a range of PMA at early MRI that included TEA, although median and interquartile ranges of PMA at MRI were all <36 weeks. Motor outcomes and the number of infants diagnosed with CP are presented in Supplementary Table 1. Few studies reported the number of infants who died as most only included infants alive at the time of outcome assessment. The percentage of infants in each cohort with adverse motor outcomes and/or a later diagnosis of CP varied considerably between studies. Cohorts with low prevalence of adverse motor outcomes frequently excluded infants with destructive brain lesions in recruitment[22, 25, 26]. Studies with a high prevalence of CP chose study participants based on the presence of defined brain lesions[23, 27-30].

Structural MRI studies

Twenty-five studies acquired early structural MRI data[21-28, 30-46]. Results of meta-analyses are presented in Table 2 and Figure 2. Sensitivity and specificity of early MRI findings in individual studies to determine later motor outcomes and/or a diagnosis of CP are presented in Table 3. Associations with adverse motor outcomes are presented in Supplementary Table 2. Early MRI global scores detected infants with a later diagnosis of CP with pooled sensitivity 100% (95%CI 86-100%) and specificity 89% (95%CI 54-100%) (total participants n=68)[27, 37, 46]. Global structural MRI scores determined adverse motor outcomes using a cut point of < -2 standard deviations (SD) on the Bayley Scales of Infant and Toddler Development 2nd edition (BSID-II; n=43), with pooled sensitivity 89% (95%CI 44-100%) and specificity 96% (95%CI 85-100%)[37, 46]. Meta-analysis of an MRI WM score to determine motor outcome on the Bayley Scales of Infant and Toddler Development 3rd edition (Bayley III; cut off < -1SD; n=240) had pooled sensitivity 33% (95% CI 20-48%) and specificity 83% (95% CI 78-88%)[31, 38].

Associations between early MRI findings and motor outcome or CP were more frequently reported than diagnostic accuracy. Five studies employed an overall score of brain injury/integrity, of which four found that poorer MRI scores were associated with adverse motor outcomes[27, 38, 40, 46]. A white matter injury (WMI) severity score[47] was associated with adverse motor outcomes in three studies[31, 34, 40] but not in two other studies[42, 43]. Greater WMI volumes in frontal, parietal and temporal, but not occipital lobes were associated with adverse motor outcomes [34]. White matter injury located in the frontal lobe was most predictive of adverse motor outcome[34]. Smaller total WM volume was associated with adverse motor outcomes[36]. Punctate WM lesions were not associated with motor outcome[21, 26], although an association between the appearance of punctate lesions and presence of CP was reported[39]. A greater degree of signal intensity change in the periventricular WM was not associated with adverse motor outcomes, in infants with otherwise normal MRI or cranial ultrasound scans[45].

Intraventricular hemorrhage (IVH) was associated with poorer motor outcomes [40, 43]. Two studies, one of which had only IVH grade I and II in their cohort[31], found no associations with adverse motor outcomes[31, 42]. Ventriculomegaly (VM) at TEA following early IVH was associated with poorer motor outcomes[26]. Increasing severity of VM on early MRI was associated with poorer motor outcome in one study[40], but another study found no associations[26], possibly because they examined VM independently of IVH.

Cerebellar hemorrhage (CBH) demonstrated no associations with motor outcomes[31, 40, 43]. Smaller cerebellar volume was associated with adverse motor outcomes when both the early and term MRI data were pooled[36]. A cerebellar score which included cerebellar injury and transcerebellar diameter was associated with adverse motor outcome[38]. Presence of periventricular hemorrhagic infarction (PVHI) was associated with adverse motor outcomes[41]. Parietal PVHI resulted in a diagnosis of CP in 50% of cases. Temporal PVHI was responsible for poorer motor outcomes than frontal PVHI[30]. In a retrospective study of infants with periventricular leukomalacia (PVL) compared to sex matched, healthy preterm controls, 23 out of 33 with PVL developed CP compared with none from the control group[23].

Smaller cortical GM volume was associated with poorer motor outcomes with early and term MRI data combined[36]. Using only early MRI data, deep GM, but not cortical GM, was associated with poorer motor outcomes[38]. Smaller deep GM volume was also associated with adverse motor outcomes when early and term MRI data were pooled[36].

In a study of infants with PVL[23], those who progressed to severe CP (Gross motor function classification system GMFCS III-V) had smaller thalamic volumes compared to no or mild CP (GMFCS I-II)[23]. A study investigating length and cross-sectional area of the corpus callosum (CC) found no association of length, total area, anterior or middle third area of the CC with motor outcomes[35]. A smaller area of the posterior third of the CC was associated with adverse motor outcomes[35].

Growth rates of cerebral volume, cortical surface area, total brain volume[25], and volume change of the hippocampus[33] between early and TEA MRI demonstrated no associations with motor outcomes. A scaling exponent of cortical surface area relative to cerebral volume was not associated with motor outcomes[22], however total cerebral volume was smaller in infants who progressed to poorer motor outcomes when early and term MRI data were combined[36].

Diffusion MRI studies

Sixteen studies from nine distinct cohorts acquired early diffusion MRI data[20, 23, 24, 29-33, 35, 37, 39, 41, 43, 44, 48, 49]. Sensitivity and specificity of diffusion MRI findings to determine later motor outcomes and/or CP in individual studies are presented in Table 3. Diffusion biomarkers, regions evaluated and associations with motor outcomes are presented in Supplementary Table 3. Three diffusion MRI studies reported diagnostic accuracy of their methods although the data were not suitable to be combined in a meta-analysis[20, 29, 48]. Asymmetry of the PLIC on visual inspection of diffusion

images, detected cases of hemiplegic CP with sensitivity 86% and specificity 100%[29]. An asymmetry index of FA >0.05 between left and right PLIC, identified cases of hemiplegic CP with sensitivity 100% and specificity 88%[29]. An asymmetry index of RD between left and right PLIC, detected later hemiplegic CP with sensitivity 71% and specificity 94%[29]. A second study combined MRI connectome network features with structural MRI brain injury grade and clinical data of GA at birth, PMA at MRI and gender and reported a sensitivity of 66% and specificity of 79% to determine adverse motor outcomes[20]. Importantly, the same group subsequently reported that connectome network features alone were not predictive of motor outcomes[48], suggesting that the variables of brain injury and perinatal data were responsible for the diagnostic accuracy of the initial overall model. The new study proposed a convolutional neural network framework (BrainNetCNN) which generated a predicted motor outcome score for an individual infant based on their diffusion MRI data[48]. They reported a mean absolute error between actual and predicted Bayley III motor composite score of 11%, and standard deviation of 8%[48].

Eight diffusion MRI studies reported associations between early MRI and motor outcomes[24, 31-33, 35, 37, 44, 49]. In WM tracts (CC genu and splenium, PLIC, optic radiation), lower FA was associated with poorer motor outcomes[31, 35, 37]. A small study (n=12) reported a slower change in FA between early and term MRI in infants with adverse motor outcomes[37]. Conversely, in a larger study (n=157), no associations were found with FA change between early and term MRI and later motor outcomes[31]. A single study evaluating microstructure of the cingulum found no associations between FA, MD, AD or RD and motor outcomes[44]. Poorer motor outcomes were associated with a difference in slope of FA between left and right inferior temporal lobes, where FA increases more slowly on left relative to the right[49]. No associations between FA and motor outcomes were found in superior WM structures (anterior, central or posterior)[31]. A slower increase in FA between early and term MRI in basal nuclei (thalamus, caudate and lentiform nuclei) was associated with poorer motor outcomes[31]. A single study of diffusion imaging in the hippocampus found no associations with weekly change in MD, AD, RD between early and term MRI and motor outcomes[33].

A whole brain voxel-based analysis, found a greater extent of abnormalities on MD, AD and RD in infants with poorer motor outcome compared to infants with normal motor outcomes[24]. Tract-based spatial statistics applied to MRI acquired at PMA of 30-33 weeks found that infants with poorer motor outcomes had higher AD and RD in the CC, internal and external capsule[32]. The same study evaluated subsets of infants scanned at 27-29 and 34-36 weeks PMA and found no associations between FA, AD and RD and later motor outcomes[32].

fMRI and MRS studies

One study performed fMRI for the purpose of identifying target regions for tractography[44]. An MRS study investigating N-acetylaspartate (NAA)/choline in the basal nuclei, WM tracts and superior WM found that slower increases in NAA/choline between early and term MRI were associated with poorer motor outcomes[31]. A second MRS study evaluated differences in NAA/choline and three other markers of metabolism between infants born appropriate weight for GA compared with infants who were small for GA. They found no differences between the groups in either their metabolic markers or motor outcomes[50].

Quality of included studies

The risk of bias and concerns regarding applicability of findings from individual studies were evaluated using the QUADAS-2 tool (results and scoring parameters are included as Supplementary Material B). Sources of potential risk of bias and applicability concerns were predominantly related to participant selection. Inadequate reporting of blinding of personnel involved in MRI analysis limited the ability to judge the risk of bias conferred from the index test. Few concerns regarding applicability were identified for the index test. As sufficient motor data or diagnoses of CP were part of the inclusion criteria for this systematic review, no concerns regarding applicability were identified for the reference standard. A potential risk of bias may exist for the reference standard, as almost half of the studies failed to report whether outcomes were assessed by personnel blinded to MRI findings. The majority of studies were judged as having a low risk of bias from flow and timing.

DISCUSSION

Early MRI demonstrates reasonable sensitivity and specificity to determine motor outcomes and CP in infants born preterm. Diagnostic accuracy for an outcome of CP was stronger than for adverse motor outcomes. In determining motor outcome, specificity was higher than sensitivity indicating that a normal MRI accurately identified infants who progressed to a normal motor outcome, whereas infants with an abnormal early MRI demonstrated variable motor outcomes. For determination of a later diagnosis of CP, sensitivity was higher than specificity. Global MRI scores accurately determined motor outcomes more strongly than WM MRI scores.

The studies included in meta-analyses of global MRI scores to determine motor outcomes and CP demonstrate a high risk of bias due to non-consecutively recruited samples featuring an over representation of infants with brain injury or an outcome of CP[27, 37, 46]. These weighted samples may have led to an overestimation of the diagnostic accuracy of MRI, and the results therefore need to

be interpreted with caution[51]. The studies included in meta-analysis of WM to determine motor outcome were found to be at low risk of bias in all domains, as judged with the QUADAS-2 tool[31, 38].

Early MRI findings were also associated with motor outcomes and CP. Poorer motor outcomes were associated with worse structural MRI global, deep gray matter and cerebellar scores, location, severity and volume of white matter injury, presence of intraventricular haemorrhage, ventriculomegaly, periventricular hemorrhagic infarction or periventricular leukomalacia, smaller thalamic volumes, and smaller area of the posterior third of the corpus callosum. Diffusion MRI showed lower fractional anisotropy and higher axial and radial diffusivity in the corpus callosum, PLIC, and a slower increase in FA between early and term MRI in the basal nuclei were associated with poorer motor outcomes.

Specificity was higher than sensitivity in most studies with diagnostic accuracy data of MRI to detect later motor outcomes. This indicates that early MRI performs well at ruling out future adverse motor outcomes. Lower sensitivity however, means that not all infants with abnormal MRI progress to adverse motor outcomes. This is consistent with studies of MRI at TEA[3, 10, 52]. For determination of a later diagnosis of CP, sensitivity was more frequently higher than specificity, indicating that most infants who progress to CP were identified by abnormalities on MRI. The inverse relationships between sensitivity and specificity for an outcome of CP compared with motor outcomes may in part be the result of variability in motor outcomes for preterm infants without CP[53]. The choice of cut points in the MRI scores for sensitivity and specificity calculations may also contribute to this difference. If MRI scores are dichotomized as 'any injury vs no injury', sensitivity tends to be high and specificity relatively low, while if 'normal/mild injury vs moderate/severe MRI scores' are used, sensitivity drops and specificity increases.

White matter abnormalities on early MRI had lower sensitivity and specificity than global measures of MRI abnormalities in determination of motor outcomes in this review. Key differences between the studies included in the meta-analyses need to be taken into consideration when interpreting this finding. Firstly, the sample size of the WM score meta-analysis was 240 participants compared to 43 in the global score meta-analysis. Secondly, the studies with a global MRI score used the BSID-II outcome, while the WM score studies used the Bayley III. This makes it difficult to determine if the MRI scoring system utilized was better at determination of outcome, or whether the BSID-II identifies a different group of children to those identified by the Bayley III. There is some concern that the Bayley III underestimates motor dysfunction in infants born preterm[54], leading to a tentative

use of <-1SD as the cut off in analyses, rather than the more widespread use of <-2SD with other validated tools.

If WM scores have less diagnostic accuracy than global scores of early MRI, then it is an important difference to findings with TEA MRI[10]. A systematic review found term MRI WMA had greater predictive accuracy for motor outcomes and CP compared with other brain abnormality findings[10]. Global scores include evaluation of WM; further investigation of other components of global scoring may provide insight into specific abnormalities that have greater diagnostic accuracy than WM abnormalities on early MRI.

The use of diffusion MRI is gaining momentum in preterm infant studies[11]. Age and maturation relationships between diffusion biomarkers and PMA at MRI have been demonstrated[55, 56], as well as changes between early and term MRI[57, 58]. Relatively few studies have demonstrated whether these maturational differences equate to clinically meaningful differences in outcomes. This review found poorer motor outcomes were associated with lower FA and lower AD and RD in the corpus callosum and PLIC on early MRI[31, 35, 37], and a slower increase in FA between early and term MRI in the PLIC[37], optic radiation[37] and basal nuclei[31]. Further research is required to determine if these findings are reproducible in other cohorts of preterm infants. Reporting of diagnostic accuracy is required to determine if these diffusion MRI biomarkers provide useful prognostic information which could be used to support clinical patient management.

Only five of the 30 studies in this review reported diagnostic accuracy; the majority reported associations between early MRI findings and motor outcomes and/or CP. This finding is consistent with a systematic review of MRI at TEA in preterm infants[11]. Statistical analyses in preterm study populations are challenged by small numbers in a cohort progressing to adverse motor outcomes or CP – a phenomenon called class imbalance in the data[59]. In these situations, the few infants with CP or adverse motor outcomes may be responsible for associations, and if removed, the association often no longer remains significant. This explains why a study may find significant associations between an MRI finding and motor outcome, but then have relatively poor sensitivity and specificity for determining motor outcome or CP. The clinical relevance is that diagnostic accuracy needs to be considered when using evidence of associations between MRI and outcomes to inform clinical practice.

A number of studies which reported no associations between MRI findings and motor outcomes had excluded infants with specified structural brain lesions which could be the reason no relationship

with motor outcomes was found[22, 25, 26, 41, 45]. Studies which excluded participants with structural brain lesions may be excluding the very cases that their analyses are trying to identify. The rate of CP and adverse motor outcomes are declining in preterm populations[60-62], increasing the challenge of identification of the few cases that do progress to adverse outcomes.

A variety of different brain structures were examined by the 30 studies included in this review. Few studies examined exactly the same structures or used exactly the same scoring methods, and those that did reported contradictory findings in some cases. White matter injury and IVH were two areas where some studies reported associations with motor outcome and others found no associations. This could be a sample size issue of inadequately powered studies either over- or underestimating associations with outcomes[63]. Or it could be related to sampling bias as some studies excluded infants with large structural brain lesions. Publication bias may also play a part as it is well known that negative findings are under-reported in the literature[64, 65]. Either way, adequately powered studies with rigorous methodology and representative sampling are required to replicate these early MRI findings and determine their true reproducibility.

While some meta-analyses were performed in this review, data of only a small number of studies could be combined; consequently the pooled results of sensitivity and specificity should be interpreted with caution. Heterogeneity of MRI scoring systems, variations of dichotomized scoring categories and differences in motor outcome measures utilized, limited the extent of meta-analyses that were able to be undertaken. No studies used exactly the same MRI scoring method and outcome measure and so studies were grouped broadly by whether they used an overall measure of injury or looked at WM only.

Few of the included studies were unselected, sequentially recruited samples of preterm infants, representative of preterm infant populations. Most studies were in tertiary centers with access to MRI and are likely to represent higher risk populations. Recruitment rates of eligible infants varied considerably between studies with some as low as 17%[37] and others as high as 86%[26]. Some studies reported high levels of recruitment, but only a proportion of the infants underwent imaging[49]. Some studies grouped data by first/second MRI rather than early/term MRI[21, 30, 31]. Diffusion studies frequently excluded a large number of scans due to movement artefact[24, 41, 44, 49].

Follow-up rates varied markedly between studies, a well-documented source of potential bias[66]. Some studies used presence of follow up data as part of their inclusion criteria and therefore 100% of the cohort had outcomes reported. Other studies had less than 50% follow-up so that even if the

cohort recruited was representative, analyses with outcomes were performed for a 'selected' subgroup[42, 44]. Despite these limitations, the studies included in this review provide important information on the consequences of early brain injury and development.

Clinical implications

Structural MRI has the greatest amount of evidence available and is the most clinically accessible of the modalities featured in this review. Valid and reliable scoring systems exist for early structural MRI[38, 40] which can be adopted into clinical practice. Evidence for diffusion MRI is emerging, but the complexity of analysis and interpretation precludes it from application to routine clinical settings at this time. The evidence from this review suggests that early MRI may play an important role in early identification of infants at risk of CP and adverse motor outcomes, and continued research of early MRI is warranted. MRI findings at any age need to be interpreted in context with other clinical findings.

Research implications

Future research of early MRI should include reporting of diagnostic accuracy in addition to associations between MRI findings and outcomes. Replication of published relationships between early MRI and motor outcomes is required to determine reproducibility of MRI scoring and analysis methods. Optimization of cut points may improve diagnostic accuracy of existing scoring systems. Receiver operating characteristic (ROC) curve and area under the curve (AUC) analyses could be employed to optimize cut-points. Examination of relationships with, and diagnostic accuracy for, longer term outcomes is required to understand the full range of implications of early brain injury and the value of early MRI in providing prognostic information. One potential benefit of early MRI is to select infants who may benefit from early interventions which could be commenced while the infant is still in the neonatal intensive care unit. Animal studies of very early neuroprotective therapies such as hypothermia, erythropoietin, melatonin, creatine and others are showing promise. The ability to identify infants who may benefit from these therapies is critical. Early MRI is resource intensive; measures such as clinical assessment findings or readily available bedside cranial ultrasound need to be examined for correlations with early brain injury on MRI. Diagnostic accuracy of MRI at TEA is augmented by the addition of clinical measures of motor or neurological function [4, 67, 68]. Evaluation of combinations of early clinical measures and early MRI to determine outcomes is warranted.

Strengths and Limitations

This is the first systematic review of diagnostic accuracy of early MRI to determine later adverse motor outcomes or a diagnosis of CP. It is also the first systematic review of early MRI to present meta-analyses of early MRI data to determine later outcomes in infants born preterm. Both diagnostic accuracy and results of associations between early MRI and later adverse motor outcomes or CP are synthesized and reported in this review, providing a comprehensive overview of the current reported evidence for early MRI to determine motor outcomes and CP in infants born preterm. The following limitations require consideration: only studies published in English were included in this review, potentially excluding some relevant studies published in other languages; some of the included studies pooled their early and term MRI data with no way to determine if the associations with outcome were driven by findings on the term MRI; heterogeneity of the included studies, in terms of participants, methods and outcomes, resulted in a limited ability to pool data.

Prediction of outcomes is highly desirable for both families and clinicians involved in the care of preterm infants, but statistical tests to determine positive and negative predictive values are greatly affected by the prevalence of adverse outcomes and CP in the study populations[17]. This greatly limits the ability to generalize the results beyond the population studied. Due to the heterogeneity of included studies in this review, and in particular the range of prevalence of adverse motor outcomes and CP in the study populations, no pooling of results would have been possible with positive or negative predictive values. Sensitivity and specificity were selected to evaluate diagnostic accuracy in this review as they are reporting the properties of the tests, rather than being impacted by the properties of the sample.

Conclusion

Identifying early markers of later adverse motor outcomes or CP remains a necessary and important challenge for researchers and clinicians. Early MRI has reasonable sensitivity and specificity to determine later motor outcomes and/or a diagnosis of CP in infants born preterm. The evidence for structural MRI with qualitative scoring of brain macrostructure is promising and clinically accessible. Evaluation of brain microstructure with diffusion MRI is emerging. Further research is required to refine scoring systems to optimize diagnostic and predictive accuracy, and to improve clinical utility. Reporting of diagnostic accuracy is critical to enable interpretation of relationships between early MRI findings and later motor outcomes and/or CP.

Acknowledgements: Thank you to Dr Jurgen Fripp for provision of images for Figure 1.

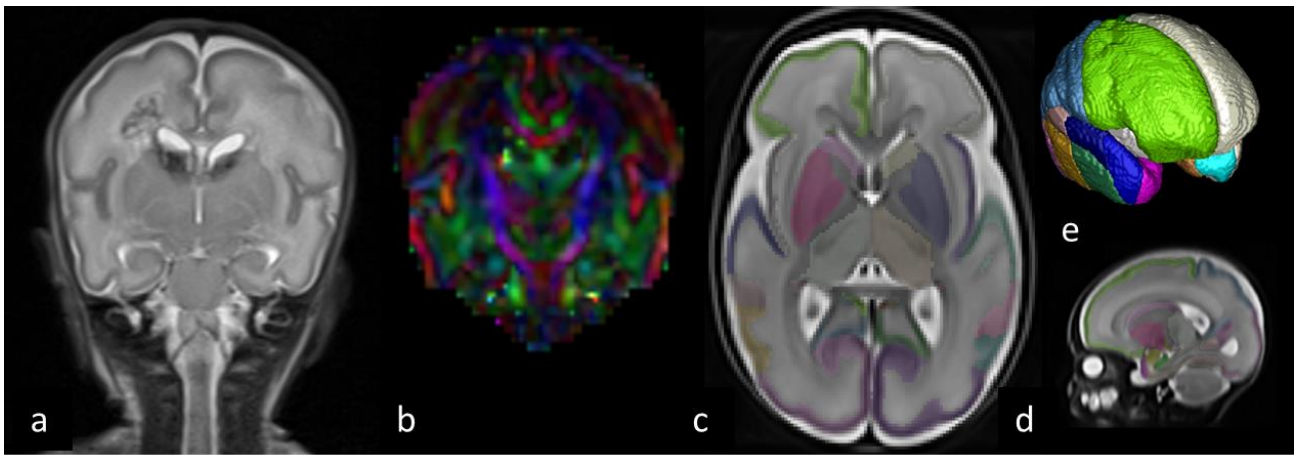


Figure 1: Examples of MRI acquisition types at approximately 32 weeks postmenstrual age: a) structural MRI evaluated qualitatively for evidence of injury; b) diffusion MRI, from which quantitative measures of brain microstructure and development can be extracted (fractional anisotropy, mean diffusivity, axial diffusivity and radial diffusivity); c-e) population 32 week equivalent atlas that can be used for subject labelling (from which diffusion MRI measures can be extracted).

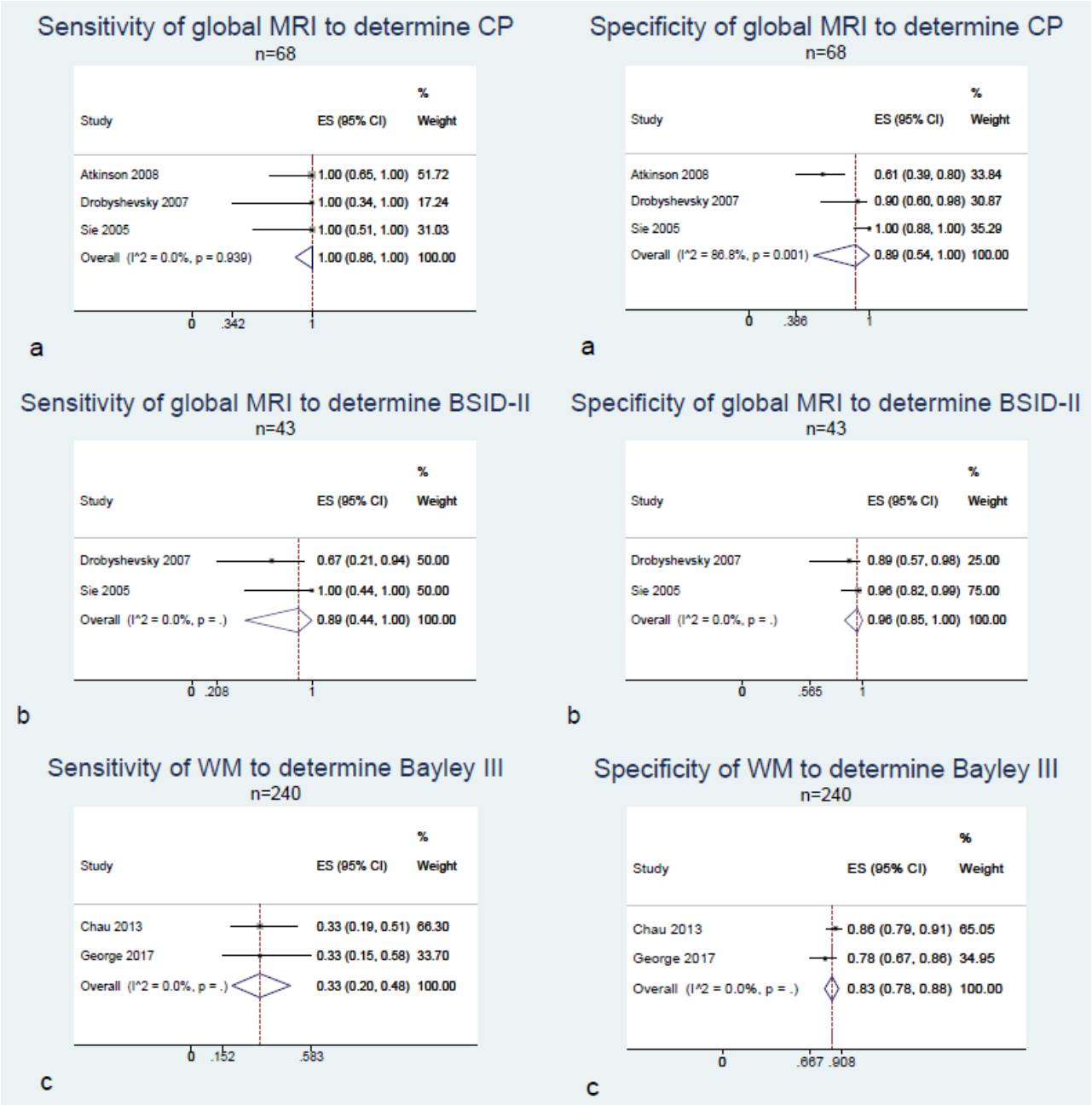
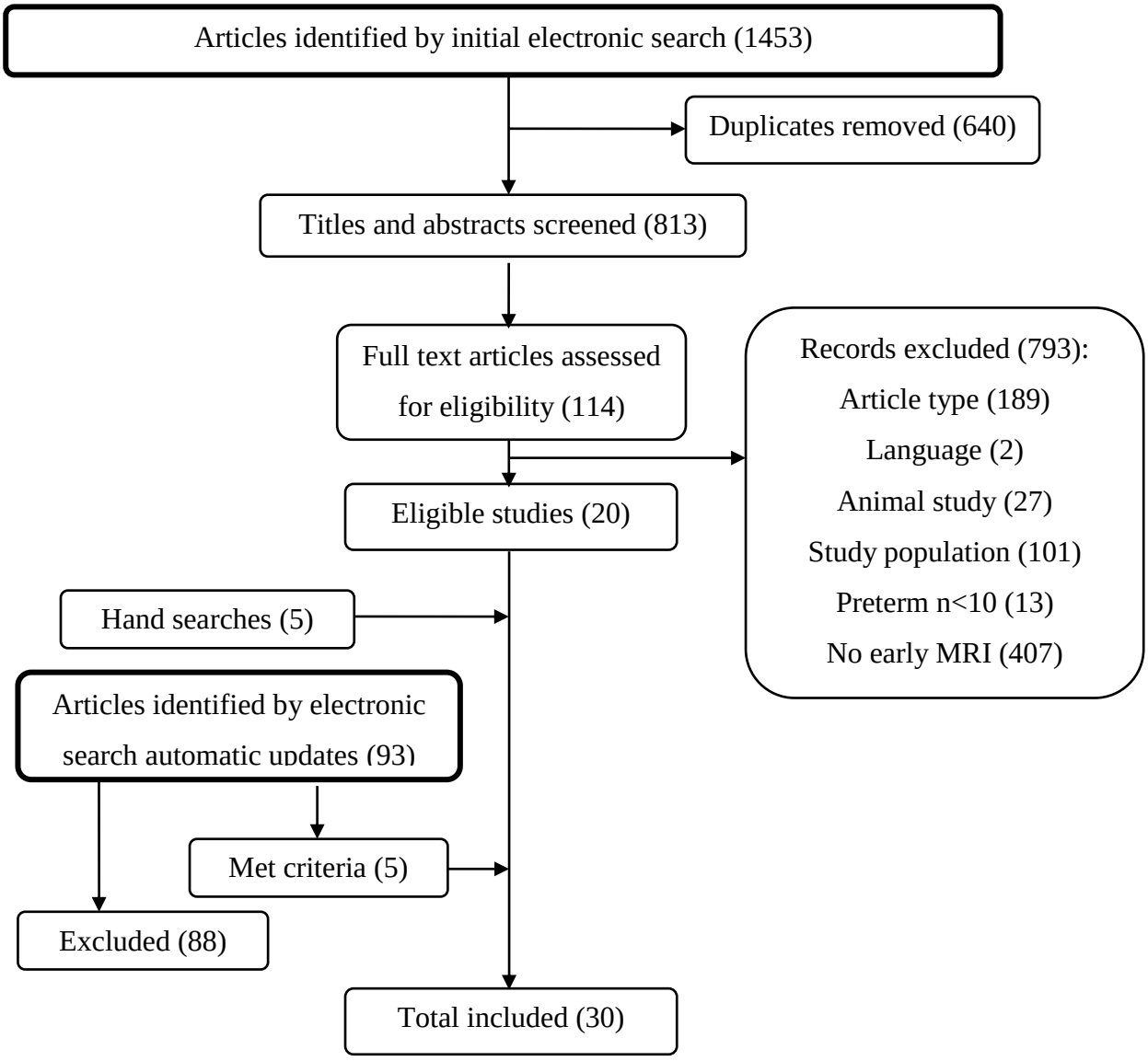


Figure 2: Results of meta-analyses. Pooled sensitivity and specificity of a) global MRI score to predict CP, b) global MRI score to predict motor outcome of < -2SD on the BSID-II, and c) MRI WM score to predict motor outcome on the Bayley III. Abbreviations: BSID-II Bayley Scales of infant and toddler development 2nd edition; Bayley III Bayley scales of infant and toddler development 3rd edition; CP cerebral palsy; ES pooled estimate; WM white matter.



Online Figure 1: Flowchart of included studies

Table 1: Population demographics of studies included in this systematic review (Studies presented in alphabetical order; by initial then subsequent publications on the same or partially overlapping cohort)

Study	**Study design**	**No. with early MRI** (no. of useable scans)	**Gestation at birth** weeks Median [IQR] or Mean[a] (SD), range	**Birth weight** grams Median [IQR] or Mean[a] (SD), range	**Sex** Male (%)	**PMA at early MRI** weeks Median [IQR] or Mean[a] (SD), range
Chau 2013[31]	P	177[b]	27.6 [25.9-29.7], 24-32	1020 [800-1285]	82 (52%)	32.1 [30.5-33.9], 27.1-46.3
Booth 2016[24]	P	55(46) con.	28.3[a]	n.s.	30 (55%)	31.3
		79 (73) exp.	27.9[a]	n.s.	38 (48%)	32.4
Brown 2015[20]	P	115 [b] (168[c])	24-32	n.s.	n.s.	27-45
Duerden 2015[32]	P	153	27.7 [26-29.7][d], 24-32	1022 [820-1281][d]	78 (51%)[d]	32 [30.4-33.7]
Duerden 2016[33]	P	138	27.7 [26-29.9], 24-32	n.s.	70 (51%)	32.3 [30.7-34]
Guo 2017[34]	P	124 No WMI	27.9 [26-30]	n.s.	69 (56%)	32.4 [30.6-34.1]
		58 WMI	28.6 [26.3-29.8]	n.s.	26 (45%)	32 [30.6-33]
Kawahara 2016[48]	P	115[b] (168[c])	24-32	n.s.	n.s.	27-45
Malavolti 2016[35]	P	193[b]	27.5 [25.8-29.5], 24.2-32	1000 [800-1270], 459-1870	n.s.	32 [30.4-34.1], 27.1-45
Zwicker 2016[36]	P	136	27.4 [25.8-29.8], 24-32	n.s.	71 (52%)	32.3 [30.8-34]
Cornette 2002[21]	R	50 [b]	30 [3.5[e]], 25-36	1280 [739 [e]], 580-3675	n.s.	n.s.
		15/50 [b] with PL	31[3.1[e]], 27-36	1320 [929 [e]], 580–3960	10 (67%)	35[3.1[e]], 29-39.7[g]
Drobyshevsky 2007 [37]	P	24 (21)	28.7 (0.4[f])[g], 24.1-30.9[g]	1244, 640-1716	n.s.	30.4 (0.41[f])[g], 25.9-32.9[g]
Dyet 2006[26]	P	119	27.6, 23-29.9	880, 370-1606	67 (56%)	2 [1-5][h]
Kapellou 2006[22]	P	119 (274[c])	26.9	n.s.	n.s.	2 [1-4][h]
Atkinson 2008[27]	P	26	28.1 (2.7)	n.s.	n.s.	<36
Rathbone 2011[25]	P	82[b] (217[c])	27.7, 23.4-29.9	950, 500-1610	43(52%)	30.6, 24.1-44
George 2017[38]	P	83	28.4 [26.6-29.3], 23.6 – 30.6	1068 (312), 494 – 1886	49(59%)	32.2 (1.3), 29.3–35.2
Kersbergen 2014[39]	R	112	28.2[a], 24.4-33.4	1158[a], 515-2100	56 (50%)	31.2[a], 26.6-34.7
Kersbergen 2015[23]	R M	33[b] with PVL	30.1, 24.7-35.4	1621[a], 705-2780	24 (73%)	33.7[a], 29.3-38
		31 con.	27.9, 25.6-31	1083[a], 565-1630	19 (61%)	31.3[a], 30.3-34.7
Miller 2005[40]	P	89	28, 24-34	n.s.[i]	47 (53%)	32 [31-33]
Pavaine 2016[41]	P	105 (85)	28.9[a], 24.7-32.9	n.s.	46 (54%)	30.4[a], 26.3-34.9
Young 2016[42]	P	105	28.6, 24.4-32.9	1142 (228)[j]	30 (58%)[j]	Within 2 weeks of birth

Roelants Van Rijn 2001[28]	n.s.	12 normal CUS 8 IVH+PI 7 cystic PVL	29.6 (1.9) 28.9 (2.3), 26.5-32.5 29.6 (2.5), 27.2-34	1230 (465) 1359 (530), 875-2520 1266 (575), 855-2410	n.s.	32.2 (0.6) 31.5 (1.3) 31.4 (2.7)
Roelants Van Rijn 2004[50]	P	26 AGA 14[b] SGA	29.2 (2.3) 30.1 (2.7)[d]	1200 (425) 675 (150)[d]	n.s.	32.1, 31.1-33.7 32.9, 31.6-33.3
Rogers 2016[49]	P	78 (50)	26.6 (1.8)	941 (246)	33 (42%)	30.4 (2.4)
Roze 2015[29]	R	23	28.9, 25.7-34.6	1200, 650-1950	9 (39%)	31.3, 29.3-36.4
Soltirovska Salamon 2014[30]	R	21[b] Frontal PVHI 13[b] Temporal PVHI	30.3[a], 28-34.4 30.3[a], 26.6-33.4	1527[a], 910-2965 1205[a], 990-2450	11 (52%) 5 (38%)	Approx. 30 Approx. 30
Tam 2016[43]	P M	60	28.2[a], 24-32	n.s.	37 (62%)	31.5 (2.3)
Cui 2016[44]	P	21 brain injury 23 con.	29.1 (1.9) 29.1 (1.5)	1278 (290) 1241 (273)	13 (62%) 13 (57%)	32.4 (1.6) 32.2 (1.5)
Van Wezel-Meijler 1999[45]	P	42	30.9 (1.6)	n.s.	n.s.	33.2 (1.4)[g]
Sie 2005[46]	P	46[kb]	31.1 (5.6), 27-41.8	1600 (490), 840-4440	35 (76%)	20 (12), 3-53[h]

Key: AGA appropriate for gestational age; CUS cranial ultrasound; IQR interquartile range; IVH+PI intraventricular hemorrhage with parenchymal involvement; M multicenter; N no; no. number; n.s. not specified; P prospective; PL punctate lesions; PVHI periventricular hemorrhagic infarction; PVL periventricular leukomalacia; R retrospective; SD standard deviation; SGA small for gestational age; WMI white matter injury; Y yes; con. control group; exp. experimental group; [a] mean; [b] sample includes some MRI performed at >36 weeks PMA; [c] represents useable scans from both early and term MRI; [d] includes data of some infants with only a term MRI; [e] standard deviation; [f] standard error; [g] reported as postconceptional age; [h] postnatal days; [i] multiple group median [IQR] reported; [j] data of n=52/105 with 2 year outcome data available; [k] sample includes 8/46 born>36 weeks GA.

Table 2: Results of meta-analyses conducted in this systematic review.

MRI score	Outcome	Number of studies	Total number of participants	Sensitivity (95%CI)	Specificity (95%CI)
sMRI global score	CP	3[27, 37, 46]	68	100 (86-100)	89 (54-100)
sMRI global score	<-2SD BSID-II	2[37, 46]	43	89 (44-100)	96 (85-100)
sMRI WM score	<-1SD Bayley III	2[31, 38]	240	33 (20-48)	83 (78-88)

Key: Data are percentages; point estimate and 95% confidence interval. Bayley III Bayley Scales of Infant and Toddler Development 3rd Edition; BSID II Bayley Scales of Infant and Toddler Development 2nd Edition; CP cerebral palsy; sMRI structural MRI; WM white matter.

Table 3: Sensitivity and specificity in individual studies of abnormal early MRI to determine adverse motor outcomes or CP. Presented as reported, or calculated from 2x2 tables of dichotomous MRI and outcome data where sufficient raw data was available.

Study	Definition of abnormal MRI	Cut off for motor outcome	n	Determination of later adverse motor outcomes		Determination of a later diagnosis of CP	
				Sensitivity	Specificity	Sensitivity	Specificity
Atkinson 2008[27]	sMRI global mod/severe injury	Griffiths DQ <-2SD	24	100 (48-100)	55 (32-77)	100 (59-100)	61 (36-83)
Chau 2013[31]	sMRI WMI mod/severe	<-1SD Bayley III	157	33 (17-53)	86 (79-91)	n.a.	n.a.
Brown 2015[20]	dMRI connectome: 3x10 network measures + IVH/WMI grade + clinical data (GA at birth, PMA at MRI, gender) & LSI[a]	<-1SD Bayley III	168 scans	66	79	n.a.	n.a.
Drobyshevsky 2007[37]	sMRI global mild-severe	<-2SD BSID-II	12	67 (9-99)	89 (52-100)	100 (16-100)	90 (56-100)
George 2017[38]	sMRI score mod/severe[b]: WM	<-1SD Bayley III	83	33 (12-62)	78 (66-87)	n.a.	n.a.
	Cortical GM			0 (0-22)	81 (70-89)		
	Deep GM			40 (16-68)	94 (86-98)		
	Cerebellum			13 (2-40)	93 (84-98)		
	Global			33 (12-62)	87 (76-94)		
	sMRI score mod/severe[b]: WM	NSMDA mild-profound dysfunction		43 (10-82)	78 (67-86)		
	Cortical GM			0 (0-41)	83 (73-91)		
	Deep GM			43 (10-82)	91 (82-96)		
	Cerebellum			29 (4-71)	93 (85-98)		
	Global			43 (10-82)	86 (76-93)		
Miller 2005[40]	sMRI WMI mod/severe	Abnormal neurodevelopmental outcome[c]	80	58 (28-85)	75 (63-85)	n.a.	n.a.
Roze 2015[29]	dMRI Visual asymmetry of PLIC		23	n.a.	n.a.	86 (42-99)	100 (76-100)
	Asymmetry index FA >0.05					100 (56-100)	88 (60-98)
	Asymmetry index RD					71 (30-95)	94 (68-100)
Sie 2005[46]	sMRI global score 5-6	BSID II <-2sd	31	100 (29-100)	96 (82-100)	100 (40-100)	100 (87-100)
	sMRI motor score 3-4			100 (29-100)	96 (82-100)	100 (40-100)	100 (87-100)

	sMRI visual score 3-4			100 (29-100)	100 (88-100)	75 (19-99)	100 (87-100)
Soltirovska Salamon 2014[30]	sMRI T as opposed to F PVHI	unfavorable outcome[d]	31	60 (32-84)	94 (70-100)	100 (3-100)	70 (51-85)

Key: Data are percentages; point estimate and 95% confidence interval. Bayley III Bayley Scales of Infant and Toddler Development 3rd Edition; BSID II Bayley Scales of Infant and Toddler Development 2nd Edition; CP cerebral palsy; dMRI diffusion MRI; DQ developmental quotient; FA fractional anisotropy; F frontal PVHI; LSI local synthetic instances (statistical method to address class imbalance in data); GA gestational age; n.a. not assessed; NSMDA Neurosensory Motor Developmental Assessment; PLIC posterior limb of the internal capsule; PMA postmenstrual age; PVHI periventricular hemorrhagic infarction; RD radial diffusivity; sMRI structural MRI; T temporal PVHI; WMI white matter injury; mod moderate; [a] early and Term MRI data included in analysis; [b] composite scoring system including brain injury and 2 dimensional measurements of brain volume; [c] defined as BSID II mental development index <70 and/or neuromotor score of 3-5[40]; [d] combination of Griffiths DQ & Neurological examination.

Supplementary Table 1: Motor outcomes and cerebral palsy in the studies included in this systematic review

Study	no.	Age at Outcome months; Median [IQR] or Mean[a] (SD), range	Measure	Group scores Median [IQR] or Mean[a] (SD), range	Normal	Mild	Moderate	Severe	Cp/ No CP	CP type (severity/ GMFCS)
Chau 2013[31]	157	18.7 [18.3-19.2]	Bayley III	107[100-110][b] 91 [88-94][c] 79 [76-82][d] 61 [58-67][e]	76 (48%)[b] 51 (32%)[c]		17 (11%)[d]	13 (8%)[e]	n.a.	
Booth 2016[24]	46 con. 73[f] exp.	18	Bayley III		46 (100%)[g] 13 (18%)[b] 37 (51%)[c]		14 (19%)[d]	9 (12%)[e]	n.a. n.a.	
Brown 2015[20]	168[f]	18	Bayley III		146 (87%)[g]		22 (13%)[h]		n.a.	
Duerden 2015[32]	150	18.7 [18.3-19.5]	Bayley III PDMS-2	99 [89-105][i] 100 [92-107][j] 93 [84-104][k] 96 [90-101][i] 96 [92-98][j] 92 [84-97][k]	84% [g]		16%[h]		n.a.	
Duerden 2016[33]	117	18.7 [18.3-19.2]	Bayley III	97 [88-107]					n.a.	
Guo 2017[34]	124[l] 30[m] 17[n] 11[o]	18.6 [18.3-19.2][l] 18.6 [18.3-19.2][m] 18.6 [18.4-20.8][n] 19 [18.3-19.8][o]	Bayley III	100 [91-107][l] 99 [88-108][m] 100 [88-103][n] 82 [75-87][o]					0/124 2/56	
Kawahara 2016[48]	168[f]	18	Bayley III		146 (87%)[g]		22 (13%)[h]		n.a.	
Malavolti 2016[35]	167	18	Bayley III	95[a] [88-107], 49-124	84 (50%)[b] 53 (32%)[c]		30 (18%)[h]		n.a.	
Zwicker 2016[36]	127	18	PDMS-2	96 [86-98]					n.a.	
Cornette 2002[21]	15[p]	19.6 (3.75), 14-26	Neuro						4 PL+ other lesion /8 PL only 2 PL +	

									other lesion	
Drobyshevsky 2007[37]	12	18-24	BSID II	50-103[q]	6[g]		3[d]	3[e]	2/10	
Dyet 2006[26]	68	23.9, 19.5-34.4	Griffiths motor Neuro	95 (21)					4/64	1[r] 2[s] 1[rs] (3[t] 1[u])
Kapellou 2006[22]	63	23.83	Griffiths	59-155[q]					n.a.	
Atkinson 2008[27]	25	22.4 (3.4)	Griffiths DQ Neuro	87 (21)	17[g]		3[d]	5[e]	7/18	
Rathbone 2011[25]	62 70	24 6.1, 5.9-6.5 years	Griffiths DQ MABC	97.11(18.06) 12.81 (7.63)					n.a.	
George 2017[38]	83	12.13 (0.3)	Bayley III NSMDA	96.96 (14.27) 179.53 (18.81)	68 (82%)[g] 76 (92%)[v]		15 (18%)[h] 7 (8%)[w]		n.a.	
Kersbergen 2014[39]	17 65	25, 22.6-31.3	Griffiths DQ Bayley III	99 (14) 108 (12)					9/103	
Kersbergen 2015[23]	61		Neuro						23[y]/38	(4[t] 2[u] 10[z] 5[†] 2[‡])
Miller 2005[40]	86	18.2 [13.1-19.2] 18.2 [13.3-21.4] 18.3 [17.4-21.5]	Composite BSID-II Neuromotor*	0 [0-1]* 1 [0-2]* 2 [1-3]*	51 (59%)[#]		22 (26%)[¨]	13 (15%)[§]	n.a.	
Pavaine 2016[41]	22[¤]			94.7 (11.9), 76-121[¤]						
	15[¥]	24	Bayley III	89.3 (11.2), 64-107[¥]					n.a.	
	4[«]			95.8 (12.7), 79-110[«]						
Young 2016[42]	52	24	Bayley III	93.4 (13.7)					n.a.	
Roelants Van Rijn 2001[28]	12[ǂ] 8[!] 7[Δ]	9-15 18	Neuro						0/12 5/3 3/1 (3 died)	3[r] 2[»] 2[s] 1[»]
Roelants Van Rijn 2004[50]	26[Ω] 14[ϟ]	24	Griffiths DQ	104 (10) 99 (9)	25 (96%)[g] 13 (93%)[g]		1 (4%)[h] 1 (7%)[h]		n.a.	
Rogers 2016[49]	65	24	Bayley III	84.6 (10.6)	9 (14%)[ẍ]	35 (52%)[ẍ]	17 (26%)[ẍ]	5 (8%)[ẍ]	n.a.	
Roze 2015[29]	23	29, 15-67	Neuro						7/16	7[r]

Soltirovska Salamon 2014[30]	21F	39, 26-48	composite		‘favorable’ 15		‘unfavorable’ 6		
			Griffiths DQ	92[a]	16 (76%)[g]		5 (24%)[h]		
			Griffiths motor		19 (90%)	2 (10%)			
			Neuro					0/21	
	11T		composite		‘favorable’ 1		‘unfavorable’ 9		
			Griffiths DQ	91[a]	6 (60%)[g]		4 (40%)[h]		
			Griffiths motor		7 (70%)		3 (30%)		
			neuro					1/9	1[r](1[t])
Tam 2016[43]	45	32.8 (3.6)	Bayley III	98.1 (14.3)				n.a.	
Cui 2016[44]	13	12	Bayley III					n.a.	
Van Wezel-Meijler 1999[45]	42	12 (0.3), 11.5-13	BSID-II	101 (15), 68-138				n.a.	
Sie 2005[46]	31◊	18	Dutch BSID II		27 (87%)[g]		1 (3%)[d]	3 (10%)[e]	
			Neuro					4/27	1[r] 2[s] 1[»]

Key: Bayley III Bayley Scales of Infant and Toddler Development 3rd Edition motor composite score; BSID II Bayley Scales of Infant and Toddler Development 2nd Edition psychomotor development index; composite combination of assessments used to define outcome categories; con. control group**;** CP cerebral palsy; DQ developmental quotient; exp. experimental group; F frontal PVHI; IQR interquartile range; GMFCS Gross Motor Function Classification System; MABC Movement Assessment Battery for Children; n.a. not assessed; Neuro neurological examination to determine presence/absence of CP; NSMDA Neurosensory Motor Developmental Assessment; PDMS-2 Peabody Developmental Motor Scales 2nd edition; PL punctate lesion; SD standard deviation; T temporal PVHI; [a] mean; [b] outcome score>100; [c] outcome score 85-100; [d] outcome score 70-85; [e] outcome score <70; [f] number represents useable scans; [g] outcome score>85; [h] outcome score <85; [i] PMA at MRI 27-29 weeks; [j] PMA at MRI 30-33 weeks; [k] PMA at MRI 34-36 weeks; [l] no white matter injury (WMI); [m] mild WMI; [n] moderate WMI; [o] severe WMI; [p] represents 15/50 with punctate lesions; [q] approximate range extracted from a figure; [r] hemiplegia; [s] diplegia; [t] GMFCS level I; [u] GMFCS level II; [v] normal or minimal dysfunction; [w] mild to profound dysfunction; [y] all infants who developed CP were in the group with cystic periventricular leukomalacia; [z] GMFCS level III; [†] GMFCS level IV; [‡] GMFCS level V; * neuromotor score[40]; [#] mental development index (MDI)>85 and normal neurologic exam; ¨ MDI 70-84 and/or motor tone/reflex abnormalities (neuromotor score 1 to 2); [§] MDI<70 and/or functional motor deficits (neuromotor score 3-5); [¤] no brain injury; [¥] mild/moderate brain injury; [«] severe brain injury; [»] quadriplegia; [ǂ] no abnormalities on cranial ultrasound; ! intraventricular hemorrhage with parenchymal involvement; [Δ] periventricular leukomalacia; [Ω] appropriate for gestational age; [ϟ] small for gestational age; [ϫ] outcome score categories normal (≥95), mild (≥80 & <95), moderate (≥65 and <80), severe (<65); ◊ subsample born <36 GA and MRI<36 weeks PMA

Supplementary Table 2: Structural MRI findings in studies included in this systematic review

Study	No.	Field Strength	Analysis	MRI Findings				
				No injury	Mild	Moderate	Severe	Poorer motor outcome is associated with
Chau 2013[31]	157[a]	1.5T	WMI	109(69%)	20(13%)	18(12%)	10 (6%)	Severe WMI
			IVH	85(54%)	71(43%)[b]	0	0	No associations
			CBH	132 (84%)		25(16%)		No associations
			PVHI	153 (97%)		4(3%)		n.a.
Booth 2016[24]	46 con. 73 exp.	1.5T	WMI	46 (100%) 44 (60%)	0 9 (12%)	0 14 (19%)	0 6 (8%)	n.a.
Duerden 2015[32]	157 scans[a]	1.5T	WMI	132 scans (84%)		25 scans (16%)		
			IVH	85 scans (54%)	68 scans (43%)[b]	4 scans (3%)[c]		n.a.
			CBH	139 scans (89%)	18 scans (11%)			
Duerden 2016[33]	138	1.5T	IVH	81 (59%)	57 (41%)[b]	0	0	n.a.
			CBH	118 (86%)		20 (14%)		n.a.
			Hippocampus Vol.					No association with hippocampal growth from early to TEA MRI[d]
Guo 2017[34]	182	1.5T	WMI	124 (68%)		58 (32%)		Severe WMI
			WMI vol.					Greater WMI volumes
			WMI location					i) greater WMI volumes in frontal, parietal, & temporal but not occipital lobes ii) Frontal lobe WMI most predictive of adverse motor outcome
			IVH	105 (58%)	72 (40%)[b]	5 (3%)[c]		n.a.
			CBH	160 (88%)		21 (12%)		n.a.
			VM	143 (79%)		39 (21%)		n.a.
Malavolti 2016[35]	193[a]	1.5T	WMI	133 (69%)	48 (25%)		12 (6%)	n.a.
			IVH	100 (52%)	85 (44%)[b]	0	8 (4%)[e]	n.a.
			CC area					i) Smaller area of posterior third of CC[d] ii) No association with total area of CC or anterior or middle third area[d]

			CC length				No associations
Zwicker 2016[36]	136	1.5T	WMI	95 (70%)	41 (30%)		n.a.
			IVH	70 (51%)	66 (49%)		n.a.
			CBH	114 (84%)	22 (16%)		n.a.
			Cerebral, WM, cortical GM, deep GM, cerebellar vol.				Smaller cerebral, WM, cortical GM, deep GM, & cerebellar volumes[d]
Cornette 2002[21]	15[af]	1.5T	PL		8/15 PL only ; 7/15 PL + other major lesion		Additional lesions & not punctate lesions alone
Drobyshevsky 2007[37]	21	n.s.	IVH	12 (57%)	6 (29%)[b]	2 (10%)[g] 1 (5%)[e]	n.a.
			VM	12 (57%)	9 (43%)		n.a.
			PVL	20 (95%)	1(5%)		n.a.
Dyet 2006[26]	119	1.0 or 1.5T	IVH	95 (80%)	24 (20%)		i. Normal early MRI scans (infants with PVHI, CBH, BG & thalamus abnormalities excluded from analysis)
			CBH	111 (93%)	8 (7%)		ii. No association with number of abnormalities
			PVHI	117 (98%)	2 (2%)		iii. No association with VM or PL
			PL	106 (89%)	13 (11%)		iv. IVH + subsequent VM
			VM	83 (70%)	36 (30%)		v. PVHI, CBH, BG & thalamus abnormalities relationships with motor outcome not assessed
			BG & thalamus	112 (94%)	7 (6%)		
Kapellou 2006[22]	113	1.0 or 1.5T	Scaling exponent of cortical surface area relative to Cerebral vol.				No associations
Atkinson 2008[27]	26	1.0T	Total brain injury score[h]	8	8	10	Worse MRI scores[i]
Rathbone 2011[25]	82	1 T	Growth rates: cerebral vol.				No associations[d]
			cortical surface area				No associations[d]
			total brain vol.				No associations[d]
George 2017[38]	83	3T	IVH	79 (95%)	4 (5%)		n.a.

			PVL	81 (98%)		2 (2%)		n.a.
			MRI scores[j]: WM					No associations
			Cortical GM					No associations
			Deep GM					worse deep GM total score
			Cerebellum					worse cerebellar total score
			Global score					worse global total score
Kersbergen 2014[39]	112	1.5 or 3T	IVH	56 (50%)	22 (20%)[b]	12 (11%)[g]	22 (20%)[e]	n.a.
			CBH	105 (94%)		7 (6%)		n.a.
			Cystic PVL	111 (99%)		1 (1%)		n.a.
			PVHI	87 (78%)		25 (22%)		n.a.
			PWML no., appearance, location	21 (19%)		91 (81%)		No association with PWML appearance & lesion load (no.) CP outcome associated with appearance of PWML
Kersbergen 2015[23]	64	1.5 or 3T	Thalamic vol. thalamic vol. corrected for Total brain vol.					Smaller thalamic volumes (severe CP GMFCS III-V compared to no/mild CP)[d]
Miller 2005[40]	86	1.5T	Global	54 (63%)		32 (37%)		Mod/severe abnormalities on MRI
			WMI	39 (49%)	17(21%)	21(26%)	3(4%)	Increasing severity of WMI
			IVH	56 (65%)	24 (28%)[b]	6 (7%)[c]		Increasing severity of IVH
			CBH	77 (90%)		9 (10%)		No associations
			VM	62 (72%)	7 (8%)	7 (8%)		Increasing severity of VM
Pavaine 2016[41]	85	1.5T	Global brain injury	41 (48%)	34 (40%)		10 (12%)	No associations
			IVH	58 (68%)	15 (18%)[b]	12 (14%)[g]		n.a.
			PVHI	82 (96%)		3 (4%)		Presence of PVHI
			PWML	52 (61%)	24 (28%)[k]	2 (2%)[l]	7 (8%)[m]	n.a.
Young 2016[42]	52[n]	n.s.	WMI	35 (67%)	13 (25%)		4 (8%)	No associations
			IVH	33 (63%)	7 (14%)[b]	12 (23%)[c]		No associations
Roelants Van Rijn 2001[28]	27	1.5T	IVH	17 (63%)		10 (37%)		n.a.
			PVL	20 (74%)		7 (26%)		n.a.
Soltirovska	21[a]	1.5 or 3T	Frontal PVHI					Temporal rather than frontal PVHI

Salamon 2014[30]	13[a]		Temporal PVHI					
Tam 2016[43]	60	1.5T	WMI	44 (73%)	7 (12%)	9 (15%)		No associations
			IVH	44 (73%)	9 (15%)[b]	7 (12%)[c]		Grade 3-4 IVH
			CBH	54 (90%)		6 (10%)		No associations
Cui 2016[44]	21[o]	3T	WMI	7 (33%)	8 (38%)	3 (14%)	3 (14%)	n.a.
			IVH	5 (24%)	10 (48%)	2 (9%)	4 (19%)	n.a.
Van Wezel-Meijler 1999[45]	42	1.5T	Periventricular WM SI	19 (45%)			23 (55%)	No associations
Sie 2005[46]	31[p]	1.5T	MRI scores: General[q]	5 (16%)	16 (52%)	9 (29%)	1(3%)	Worse general scores
			Motor	17 (55%)	10 (32%)	2 (7%)	2 (7%)	Worse motor scores
			Visual	15 (48%)	13 (42%)	2 (7%)	1 (3%)	Worse visual scores

Key: BG basal ganglia; CBH cerebellar hemorrhage; CC corpus callosum; con. control group; CP cerebral palsy; exp. experimental group; GM gray matter; IVH intraventricular hemorrhage; n.a. not assessed; PL punctate lesion; PVHI periventricular hemorrhagic infarction; PWML punctate white matter lesion; SI signal intensity; VM ventriculomegaly; WM white matter; WMI white matter injury; vol. volume; [a] sample includes some early MRI performed at >36 weeks PMA; [b] IVH grade 1/2[69]; [c] IVH grade 3/4[69]; [d] early and Term MRI data included in analysis; [e] grade 4 IVH[69]; [f] represents 15/50 with punctate lesions; [g] IVH grade 3[69]; [h] MRI score is a combination of early and TEA MRI findings; [i] calculated from raw data available in publication; [j] scoring system including brain injury and 2 dimensional measurements of brain volume; [k] isolated PWML; [l] linearly arranged PWML; [m] confluent PWML; [n] MRI data of 52/105 with outcome data available; [o] brain injury group only (control group had no structural abnormalities); [p] subsample born <36 GA and MRI<36 weeks PMA; [q] general score classifications normal (1), mild (2&3), moderate (4&5), severe (6).

Supplementary Table 3: Diffusion MRI findings in studies included in this systematic review

Study	Number	Biomarker	Region	Poorer motor outcome is associated with
Chau 2013[31]	157[a]	FA	WM tracts (CC genu & splenium, PLIC, OR)	lower FA (when cohort grouped by motor outcome); no association with FA change between early & term[b]
			Basal nuclei (thalamus, caudate, lentiform nuclei)	slower FA increase between early & term[b]
			Superior WM (anterior, central, posterior)	no associations
Booth 2016[24]	91 scans[ac] (80 normal & 11 abnormal outcome[d])	extent of FA/MD/AD/RD abnormalities measured by STEAM	abnormalities identified for each individual	greater extent of abnormalities on MD, AD, RD (trend towards greater extent of abnormalities on FA)
Brown 2015[20]	115[a] (168 scans)	Connectome: 3x10 network measures + IVH/WMI grade + clinical data (GA at birth, PMA at MRI, gender)	Whole brain network	See Table 5 for sensitivity & specificity
Duerden 2015[32]	22 @ 27-29w PMA 93 @ 30-33w PMA 32 @ 34-36w PMA	FA, AD, RD	whole brain; CC	27-29 weeks: no associations; 30-33 weeks: poorer fine motor; lower AD, RD in CC, IC, EC to cerebral peduncles; no association with FA; no association with gross and total motor scores 34-36 weeks: no associations
Duerden 2016[33]	117	MD, AD, RD	hippocampus	no associations with weekly change in MD, AD, RD between early and term MRI[b]
Kawahara 2016[48]	115[a] (168 scans)	Connectome: streamline number	Whole brain network	Prediction of motor outcome: absolute error between actual and predicted Bayley III motor composite score: mean 11% (standard deviation 8%)
Malavolti 2016[35]	167[a]	FA	CC genu & splenium	lower FA[b]
Drobyshevsky 2007[37]	12	FA, MD	21 regions distributed over brain; of note: PLIC	lower FA (30 weeks); slower FA change between early & term[b]
			occipital WM	slower FA change between early & term[b]
Kersbergen 2014[39]	27	ADC – visual evidence of	whole brain	n.a.

		restricted diffusion in PWML		
Kersbergen 2015[23]	29	FA, MD, AD, RD	CST, PLIC	n.a.
Pavaine 2016[41]	85	FA, MD, AD, RD	PLIC, OR	not reported
Rogers 2016[49]	38	FA, MD, AD, RD slopes of FA & MD using serial scans	ALIC, PLIC, OR, CC, cingulum bundle, centrum semiovale, frontal lobe in the forceps minor & subcortical WM of superior temporal lobe, inferior temporal lobe & orbitofrontal region.	Difference in slope of FA between left & right inferior temporal lobe, where FA increases more slowly on left relative to the right[b]
Roze 2015[29]	23 with PVHI	asymmetry of FA, MD, AD, RD; visual asymmetry	PLIC	See Table 5 for sensitivity & specificity
Soltirovska Salamon 2014[30]	5 with temporal PVHI	Visual inspection	OR	n.a.
Tam 2016[43]	45	FA, MD, AD, RD	Whole brain PLIC, OR	n.a.
Cui 2016[44]	13	FA, MD, RD, AD	cingulum	no associations

Key: AD axial diffusivity; ADC apparent diffusion coefficient; CC corpus callosum; CST cortico-spinal tract; EC external capsule; FA fractional anisotropy; GM gray matter; IC internal capsule; IVH intraventricular hemorrhage; MD mean diffusivity; n.a. not assessed; OR optic radiation; PLIC posterior limb of the internal capsule; PMA postmenstrual age; PVHI periventricular hemorrhagic infarction; PWML punctate white matter lesions; RD radial diffusivity; STEAM Statistical Template Estimation for Abnormality Mapping; WM white matter; WMI white matter injury; [a] sample includes some early MRI performed at >36 weeks PMA; [b] early and term MRI data included in analysis; [c] experimental group only; [d] n=22 infants with 'borderline outcome' excluded from analysis.

Supplementary Material A - Detailed systematic review search strategy

Pubmed (447)
preterm OR “premature infant” OR “infant, premature"[MeSH Terms]
mri OR mr[tiab] OR “magnetic resonance imaging” OR "magnetic resonance imaging"[MeSH Terms] OR “magnetic resonance” OR dti[tiab] OR diffusion
motor[tiab] OR "Motor Activity"[Mesh] OR neuromotor OR bayley OR aims OR nsmda OR mabc OR Griffith[tiab] OR Griffiths[tiab] OR "cerebral palsy"[MeSH Terms] OR "cerebral palsy" OR cp[tiab]

Scopus (192)
premature infant OR preterm OR prematurity
mri OR mr OR “magnetic resonance imaging” OR “magnetic resonance” OR dti OR diffusion
motor OR 'motor activity' OR neuromotor OR bayley OR aims OR nsmda OR mabc OR griffith* OR peabody OR "cerebral palsy" OR cp

Cinahl (117)
preterm OR “premature infant” OR MH "Infant, Premature"
mri OR TI mr OR AB mr OR “magnetic resonance imaging” OR MH "Magnetic Resonance Imaging+" OR “magnetic resonance” OR TI dti OR AB dti OR diffusion
TI motor OR AB motor OR MH "Motor Skills+" OR MH "Motor Skills Disorders" OR neuromotor OR bayley OR aims OR nsmda OR mabc OR Griffith* OR Peabody OR MH "Cerebral Palsy" OR "cerebral palsy" OR TI cp OR AB cp

Embase (666)
'prematurity'/exp OR “premature infant” OR preterm
mri OR mr:ti,ab OR “magnetic resonance imaging” OR 'nuclear magnetic resonance imaging'/exp OR “magnetic resonance” OR dti:ti,ab OR diffusion
motor:ti:ab OR 'motor activity'/exp OR neuromotor OR bayley OR aims OR nsmda OR mabc OR Griffith* OR Peabody OR "cerebral palsy" OR 'cerebral palsy'/exp

Cochrane (31)
premature infant OR preterm OR prematurity
mri OR mr OR “magnetic resonance imaging” OR “magnetic resonance” OR dti OR diffusion
motor OR 'motor activity' OR neuromotor OR bayley OR aims OR nsmda OR mabc OR Griffith* OR Peabody OR "cerebral palsy"

Supplementary Material B: Assessment of the risk of bias and concerns regarding applicability, evaluated using the QUADAS-2 tool[19] for studies included in this systematic review

	Risk of bias				Applicability concerns		
	Patient selection	**Index test (MRI)**	**Reference standard (motor outcome)**	**Flow and timing**	**Patient selection**	**Index test**	**Reference standard**
Study	Could selection of study participants have introduced bias? Was a consecutive or random sample enrolled? Did study avoid inappropriate exclusions?	Could conduct or interpretation of MRI have introduced bias? Blinded scoring of MRI? Were sensitivity & specificity cut point criteria pre-specified?	Could outcome assessment, its conduct, or its interpretation have introduced bias? Were outcome assessors blind to MRI findings?	Could patient flow have introduced bias? Did all participants receive the same outcome assessment? Were all patients included in analysis?	Are there concerns that individual study participants do not match review question?	Are there concerns that the MRI, its conduct, or interpretation differ from the review question?	Are there concerns that the target condition as defined by the reference standard does not match the review question?
Chau 2013[31]	+	+	+	+	—	+	+
Booth 2016[24]	—	+	+	+	—	+	+
Brown 2015[20]	?	?	?	+	—	—	+
Duerden 2015[32]	+	+	+	+	+	+	+
Duerden 2016[33]	+	?	+	+	+	+	+
Guo 2017[34]	+	+	+	+	+	+	+
Kawahara 2016[48]	—	?	?	+	—	+	+
Malavolti 2016[35]	+	+	+	+	—	—	+
Zwicker 2016[36]	+	+	+	+	+	—	+
Cornette 2002[21]	—	+	—	+	—	+	+
Drobyshevsky 2007[37]	—	+	+	—	—	+	+
Dyet 2006[26]	+	?	+	—	+	+	+
Kapellou 2006[22]	+	?	+	—	—	+	+
Atkinson 2008[27]	—	?	+	+	—	+	+
Rathbone 2011[25]	+	?	?	—	—	+	+

George 2017[38]	+	+	+	+	+	+	+
Kersbergen 2014[39]	—	?	?	—	—	+	+
Kersbergen 2015[23]	—	+	?	+	—	+	+
Miller 2005[40]	+	+	?	+	+	+	+
Pavaine 2016[41]	—	?	?	—	+	+	+
Young 2016[42]	—	?	?	+	+	+	+
Roelants Van Rijn 2001[28]	—	+	?	+	+	+	+
Roelants Van Rijn 2004[50]	—	?	?	+	—	+	+
Rogers 2016[49]	+	+	+	+	+	+	+
Roze 2015[29]	—	+	?	+	—	+	+
Soltirovska Salamon 2014[30]	—	?	?	+	—	+	+
Tam 2016[43]	+	+	+	—	+	+	+
Cui 2016[44]	?	?	?	—	+	—	+
Van Wezel-Meijler 1999[45]	—	+	+	+	+	+	+
Sie 2005[46]	—	?	?	—	+	+	+

+ low risk; — high risk; ? unclear risk

Supplementary Material B continued: QUADAS-2 methodological evaluation of included studies[19]

Domain 1: Participant selection

A. Risk of bias

Was a consecutive or random sample of participants enrolled?

YES: if the articles clearly stated that a consecutive or random samples was enrolled;

NO: if it was clear that this was not the case (e.g. if a study included participants with MRI for clinical reasons' (convenience sample), MRI only performed if abnormalities detected on CUS;

UNCLEAR: in other cases where it was not clear if consecutive or random samples were enrolled.

Did the study avoid inappropriate exclusions?

Inappropriate exclusions included: large number of scans excluded due to movement artefact (dMRI). Acceptable exclusions included: congenital abnormality/malformation, chromosomal abnormality, congenital infections, medical instability as reason for no MRI, geographical boundaries, language, large lesion such as PVL/PVHI (due to very low prevalence, and clear risk of outcome already established).

YES: if inappropriate exclusions were not found in the included study,

NO: if reasons for inappropriate exclusion were found.

UNCLEAR: if there was no description of the inclusion and exclusion criteria and inappropriate exclusion could not be ascertained.

Could the selection of participants have introduced bias?

LOW RISK: if all questions were scored "YES", or a maximum of one question with unclear.

HIGH RISK: if at least one question was scored as "NO".

UNCLEAR RISK: if at least two questions were scored as "UNCLEAR" and one as "NO".

B. Concerns regarding applicability

Was there concern that the included participants did not match the review question?

LOW CONCERN: if all included participants were preterm born with MRI before 36 weeks PMA. If sufficient data were reported so that data from only preterm born infants with MRI<36 weeks could be calculated and reported in SR. If motor outcomes and CP were appropriately represented in the cohort.

HIGH CONCERN: if sample included participants that were born >36 weeks GA or with PMA at MRI>36 weeks. Or if sample had an over-representation of brain injury or adverse outcomes/CP.

UNCLEAR CONCERN: if it is unclear whether the study fulfilled either the criteria for low concern or for high concern.

Domain 2: Index test

A. Risk of bias

Were the MRI results interpreted without knowledge of the results of the motor outcome?

Were sensitivity & specificity cut point criteria pre-specified?

YES: if people performing the MRI scoring were blinded to the results outcome assessments, or if the MRI scoring/analysis method was objective;

NO: if people performing the MRI scoring had knowledge of the results of outcome assessments;

UNCLEAR: if the study did not explicitly describe how the MRI was conducted, scored or interpreted.

Could the conduct or interpretation of the MRI have introduced bias?

LOW RISK: if the MRI scoring was performed blinded to the results of the outcome assessment.

HIGH RISK: if there was prior knowledge of the results of the outcome assessment.

UNCLEAR RISK: if there was no clear description of blinding of MRI scoring/analysis.

B. Concerns regarding applicability

Was there concern that the index test (MRI acquisition and scoring), its conduct, or interpretation differed from the review question?

LOW CONCERN: if the MRI scoring system was designed for use in preterm infants with the intention of identifying abnormalities which might be related to later motor outcomes, and if only early MRI data was used in analysis. Only exception here was if analysis method was examining change between early and term MRI.

HIGH CONCERN: if the MRI scoring system was not designed for use in preterm infants with the intention of identifying abnormalities which might be related to later motor outcomes, or if early and term MRI data were pooled for analysis.

UNCLEAR CONCERN: if it was unclear whether the study fulfilled criteria for "low concern" or "high concern" or if the study provided limited information regarding the conduct and interpretation of the MRI.

Domain 3: Reference standard

A. Risk of bias

Describe the reference standard and how it was conducted and interpreted

Was the reference standard likely to correctly classify the target condition?

YES: if the outcome assessment was a validated assessment tool with quantitative data of motor outcome, or a structured neurological assessment to determine the presence/absence of cerebral palsy;

NO: if the test used for outcome was not validated;

UNCLEAR: if there was no description of the outcome assessment used.

Were the outcome assessment results interpreted without knowledge of the results of the MRI findings?

YES: if people performing and scoring the outcome assessments were blinded to the results of the early MRI;

NO: if people performing and scoring the outcome assessments had knowledge of the MRI results;

UNCLEAR: if the study did not explicitly describe how the outcome assessment was conducted and interpreted.

Could the reference standard, its conduct, or its interpretation have introduced bias?

LOW RISK: if the reference standard (outcome assessment) used was performed and evaluated without knowledge of the results of the index test (MRI).

HIGH RISK: if the reference standard (outcome assessment) was evaluated with the knowledge of the results of the index test (MRI).

UNCLEAR RISK: if there was no clear description of the reference standard used, how it was performed and interpreted in relation to the results of the index test.

B. Concerns regarding applicability

Was there concern that the target condition as defined by the reference standard did not match the review question?

LOW CONCERN: if motor outcomes or CP were described.

HIGH CONCERN: not applicable, as studies were only included in this systematic review if they contained quantitative motor outcome data or data of diagnosis of CP in study sample.

UNCLEAR CONCERN: if it was unclear whether the study fulfilled either the criteria for "low concern" or for "high concern".

Domain 4: Flow and timing

A. Risk of bias

Did all participants receive the same outcome assessment? Were all patients included in analysis (i.e. follow up rate)? Was there an appropriate interval between index test(s) and reference standard? Was motor outcome evaluated at ≥12 months CA? (not applicable as studies were only included if motor outcomes were assessed at ≥12 months corrected age)

YES: if ≥80% of study participants underwent outcome assessment and were included in analyses;
NO: if<80% of study participants underwent outcome assessment and were included in analyses;
UNCLEAR: if there was no description of how and when the samples for both the index text and the reference standard were collected.

Could the participant flow have introduced bias?

LOW CONCERN: if the answer to the above question was "YES" which means that >80% of participants enrolled in the study were subjected to the same reference standard and index test, and were included in the final analysis.
HIGH CONCERN: if above question was answered "NO".
UNCLEAR CONCERN: if it was unclear whether the study fulfilled either the criteria for "low concern" or for "high concern".

References

1. Krageloh-Mann I, Horber V. The role of magnetic resonance imaging in elucidating the pathogenesis of cerebral palsy: a systematic review. *Dev Med Child Neurol* 2007; **49**: 144-51.

2. Report of the Australian Cerebral Palsy Register, Birth Years 1993-2009. 2016.

3. Woodward LJ, Anderson PJ, Austin NC, Howard K, Inder TE. Neonatal MRI to predict neurodevelopmental outcomes in preterm infants. *N Engl J Med* 2006; **355**: 685-94.

4. Spittle AJ, Boyd RN, Inder TE, Doyle LW. Predicting motor development in very preterm infants at 12 months' corrected age: the role of qualitative magnetic resonance imaging and general movements assessments. *Pediatrics* 2009; **123**: 512-7.

5. de Kieviet JF, Piek JP, Aarnoudse-Moens CS, Oosterlaan J. Motor development in very preterm and very low-birth-weight children from birth to adolescence: a meta-analysis. *JAMA* 2009; **302**: 2235-42.

6. Nanba Y, Matsui K, Aida N, et al. Magnetic resonance imaging regional T1 abnormalities at term accurately predict motor outcome in preterm infants. *Pediatrics* 2007; **120**: e10-9.

7. Mirmiran M, Barnes PD, Keller K, et al. Neonatal brain magnetic resonance imaging before discharge is better than serial cranial ultrasound in predicting cerebral palsy in very low birth weight preterm infants. *Pediatrics* 2004; **114**: 992-8.

8. Spittle AJ, Cheong J, Doyle LW, et al. Neonatal white matter abnormality predicts childhood motor impairment in very preterm children. *Dev Med Child Neurol* 2011; **53**: 1000-6.

9. Bosanquet M, Copeland L, Ware R, Boyd R. A systematic review of tests to predict cerebral palsy in young children. *Dev Med Child Neurol* 2013; **55**: 418-26.

10. Van't Hooft J, van der Lee JH, Opmeer BC, et al. Predicting developmental outcomes in premature infants by term equivalent MRI: systematic review and meta-analysis. *Syst Rev* 2015; **4**: 71.

11. Parikh NA. Advanced neuroimaging and its role in predicting neurodevelopmental outcomes in very preterm infants. *Semin Perinatol* 2016; **40**: 530-41.

12. Anderson PJ, Cheong JL, Thompson DK. The predictive validity of neonatal MRI for neurodevelopmental outcome in very preterm children. *Semin Perinatol* 2015; **39**: 147-58.

13. Pandit AS, Ball G, Edwards AD, Counsell SJ. Diffusion magnetic resonance imaging in preterm brain injury. *Neuroradiology* 2013; **55 Suppl 2**: 65-95.

14. Ment LR, Hirtz D, Huppi PS. Imaging biomarkers of outcome in the developing preterm brain. *Lancet Neurol* 2009; **8**: 1042-55.

15. Plaisier A, Govaert P, Lequin MH, Dudink J. Optimal timing of cerebral MRI in preterm infants to predict long-term neurodevelopmental outcome: a systematic review. *AJNR Am J Neuroradiol* 2014; **35**: 841-7.

16. Lane A, Chuk LM, Colditz PB, Coulthard A. The MRI-compatible neonatal incubator in practice. *J Paediatr Child Health* 2013; **49**: E377-80.

17. Hanna SE. High and variable prevalence does matter in reviews of diagnostic accuracy in cerebral palsy. *Dev Med Child Neurol* 2013; **55**: 397-8.

18. Leeflang MM, Bossuyt PM, Irwig L. Diagnostic test accuracy may vary with prevalence: implications for evidence-based diagnosis. *J Clin Epidemiol* 2009; **62**: 5-12.

19. Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011; **155**: 529-36.

20. Brown CJ, Miller SP, Booth BG, et al. Prediction of motor function in very preterm infants using connectome features and local synthetic instances. *International Conference on Medical Image Computing and Computer-Assisted Intervention* 2015: 69-76.

21. Cornette LG, Tanner SF, Ramenghi LA, et al. Magnetic resonance imaging of the infant brain: anatomical characteristics and clinical significance of punctate lesions. *Arch Dis Child Fetal Neonatal Ed* 2002; **86**: F171-7.

22. Kapellou O, Counsell SJ, Kennea N, et al. Abnormal cortical development after premature birth shown by altered allometric scaling of brain growth. *PLoS Med* 2006; **3**: e265.

23. Kersbergen KJ, de Vries LS, Groenendaal F, et al. Corticospinal Tract Injury Precedes Thalamic Volume Reduction in Preterm Infants with Cystic Periventricular Leukomalacia. *J Pediatr* 2015; **167**: 260-8 e3.

24. Booth BG, Miller SP, Brown CJ, et al. STEAM - Statistical Template Estimation for Abnormality Mapping: A personalized DTI analysis technique with applications to the screening of preterm infants. *Neuroimage* 2016; **125**: 705-23.

25. Rathbone R, Counsell SJ, Kapellou O, et al. Perinatal cortical growth and childhood neurocognitive abilities. *Neurology* 2011; **77**: 1510-7.

26. Dyet LE, Kennea N, Counsell SJ, et al. Natural history of brain lesions in extremely preterm infants studied with serial magnetic resonance imaging from birth and neurodevelopmental assessment. *Pediatrics* 2006; **118**: 536-48.

27. Atkinson J, Braddick O, Anker S, et al. Cortical vision, MRI and developmental outcome in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2008; **93**: F292-7.

28. Roelants-van Rijn AM, Groenendaal F, Beek FJ, Eken P, van Haastert IC, de Vries LS. Parenchymal brain injury in the preterm infant: comparison of cranial ultrasound, MRI and neurodevelopmental outcome. *Neuropediatrics* 2001; **32**: 80-9.

29. Roze E, Benders MJ, Kersbergen KJ, et al. Neonatal DTI early after birth predicts motor outcome in preterm infants with periventricular hemorrhagic infarction. *Pediatr Res* 2015; **78**: 298-303.

30. Soltirovska Salamon A, Groenendaal F, van Haastert IC, et al. Neuroimaging and neurodevelopmental outcome of preterm infants with a periventricular haemorrhagic infarction located in the temporal or frontal lobe. *Dev Med Child Neurol* 2014; **56**: 547-55.

31. Chau V, Synnes A, Grunau RE, Poskitt KJ, Brant R, Miller SP. Abnormal brain maturation in preterm neonates associated with adverse developmental outcomes. *Neurology* 2013; **81**: 2082-9.

32. Duerden EG, Foong J, Chau V, et al. Tract-based spatial statistics in preterm-born neonates predicts cognitive and motor outcomes at 18 months. *AJNR Am J Neuroradiol* 2015; **36**: 1565-71.

33. Duerden EG, Guo T, Dodbiba L, et al. Midazolam dose correlates with abnormal hippocampal growth and neurodevelopmental outcome in preterm infants. *Ann Neurol* 2016; **79**: 548-59.

34. Guo T, Duerden EG, Adams E, et al. Quantitative assessment of white matter injury in preterm neonates: Association with outcomes. *Neurology* 2017; **88**: 614-22.

35. Malavolti AM, Chau V, Brown-Lum M, et al. Association between corpus callosum development on magnetic resonance imaging and diffusion tensor imaging, and neurodevelopmental outcome in neonates born very preterm. *Dev Med Child Neurol* 2017; **59**: 433-40.

36. Zwicker JG, Miller SP, Grunau RE, et al. Smaller Cerebellar Growth and Poorer Neurodevelopmental Outcomes in Very Preterm Infants Exposed to Neonatal Morphine. *J Pediatr* 2016; **172**: 81-7 e2.

37. Drobyshevsky A, Bregman J, Storey P, et al. Serial diffusion tensor imaging detects white matter changes that correlate with motor outcome in premature infants. *Dev Neurosci* 2007; **29**: 289-301.

38. George JM, Fiori S, Fripp J, et al. Validation of an MRI Brain Injury and Growth Scoring System in Very Preterm Infants Scanned at 29- to 35-Week Postmenstrual Age. *AJNR Am J Neuroradiol* 2017: May 18. doi: 0.3174/ajnr.A5191 [Epub ahead of print].

39. Kersbergen KJ, Benders MJ, Groenendaal F, et al. Different patterns of punctate white matter lesions in serially scanned preterm infants. *PLoS One* 2014; **9**: e108904.

40. Miller SP, Ferriero DM, Leonard C, et al. Early brain injury in premature newborns detected with magnetic resonance imaging is associated with adverse early neurodevelopmental outcome. *J Pediatr* 2005; **147**: 609-16.

41. Pavaine J, Young JM, Morgan BR, Shroff M, Raybaud C, Taylor MJ. Diffusion tensor imaging-based assessment of white matter tracts and visual-motor outcomes in very preterm neonates. *Neuroradiology* 2016; **58**: 301-10.

42. Young JM, Morgan BR, Powell TL, et al. Associations of Perinatal Clinical and Magnetic Resonance Imaging Measures with Developmental Outcomes in Children Born Very Preterm. *J Pediatr* 2016; **170**: 90-6.

43. Tam EW, Chau V, Barkovich AJ, et al. Early postnatal docosahexaenoic acid levels and improved preterm brain development. *Pediatr Res* 2016; **79**: 723-30.

44. Cui J, Tymofiyeva O, Desikan R, et al. Microstructure of the Default Mode Network in Preterm Infants. *AJNR Am J Neuroradiol* 2017; **38**: 343-8.

45. Van Wezel-Meijler G, Van Der Knaap MS, Oosting J, et al. Predictive value of neonatal MRI as compared to ultrasound in premature infants with mild periventricular white matter changes. *Neuropediatrics* 1999; **30**: 231-8.

46. Sie LT, Hart AA, van Hof J, et al. Predictive value of neonatal MRI with respect to late MRI findings and clinical outcome. A study in infants with periventricular densities on neonatal ultrasound. *Neuropediatrics* 2005; **36**: 78-89.

47. Miller SP, Cozzio CC, Goldstein RB, et al. Comparing the diagnosis of white matter injury in premature newborns with serial MR imaging and transfontanel ultrasonography findings. *AJNR Am J Neuroradiol* 2003; **24**: 1661-9.

48. Kawahara J, Brown CJ, Miller SP, et al. BrainNetCNN: Convolutional neural networks for brain networks; towards predicting neurodevelopment. *Neuroimage* 2017; **146**: 1038-49.

49. Rogers CE, Smyser T, Smyser CD, Shimony J, Inder TE, Neil JJ. Regional white matter development in very preterm infants: perinatal predictors and early developmental outcomes. *Pediatr Res* 2016; **79**: 87-95.

50. Roelants-van Rijn AM, van der Grond J, Stigter RH, de Vries LS, Groenendaal F. Cerebral structure and metabolism and long-term outcome in small-for-gestational-age preterm neonates. *Pediatr Res* 2004; **56**: 285-90.

51. Whiting P, Rutjes AW, Reitsma JB, Glas AS, Bossuyt PM, Kleijnen J. Sources of variation and bias in studies of diagnostic accuracy: a systematic review. *Ann Intern Med* 2004; **140**: 189-202.

52. Slaughter LA, Bonfante-Mejia E, Hintz SR, Dvorchik I, Parikh NA. Early Conventional MRI for Prediction of Neurodevelopmental Impairment in Extremely-Low-Birth-Weight Infants. *Neonatology* 2016; **110**: 47-54.

53. Williams J, Lee KJ, Anderson PJ. Prevalence of motor-skill impairment in preterm children who do not develop cerebral palsy: a systematic review. *Dev Med Child Neurol* 2010; **52**: 232-7.

54. Anderson PJ, Burnett A. Assessing developmental delay in early childhood - concerns with the Bayley-III scales. *Clin Neuropsychol* 2017; **31**: 371-81.

55. Rose J, Vassar R, Cahill-Rowley K, Guzman XS, Stevenson DK, Barnea-Goraly N. Brain microstructural development at near-term age in very-low-birth-weight preterm infants: an atlas-based diffusion imaging study. *Neuroimage* 2014; **86**: 244-56.

56. Rasmussen JM, Kruggel F, Gilmore JH, et al. A novel maturation index based on neonatal diffusion tensor imaging reflects typical perinatal white matter development in humans. *Int J Dev Neurosci* 2017; **56**: 42-51.

57. Schneider J, Kober T, Bickle Graz M, et al. Evolution of T1 Relaxation, ADC, and Fractional Anisotropy during Early Brain Maturation: A Serial Imaging Study on Preterm Infants. *AJNR Am J Neuroradiol* 2016; **37**: 155-62.

58. Braga RM, Roze E, Ball G, et al. Development of the Corticospinal and Callosal Tracts from Extremely Premature Birth up to 2 Years of Age. *PLoS One* 2015; **10**: e0125681.

59. Japkowicz N, Stephen S. The class imbalance problem: A systematic study. *Intelligent data analysis* 2002; **6**: 429-49.

60. Reid SM, Meehan E, McIntyre S, et al. Temporal trends in cerebral palsy by impairment severity and birth gestation. *Dev Med Child Neurol* 2016; **58 Suppl 2**: 25-35.

61. Sellier E, Platt MJ, Andersen GL, et al. Decreasing prevalence in cerebral palsy: a multi-site European population-based study, 1980 to 2003. *Dev Med Child Neurol* 2016; **58**: 85-92.

62. Harris SL, Austin NC, Battin M, et al. In-hospital morbidity and brain metrics of preterm neonates born 1998-2009. *N Z Med J* 2016; **129**: 94-107.

63. Button KS, Ioannidis JP, Mokrysz C, et al. Power failure: why small sample size undermines the reliability of neuroscience. *Nat Rev Neurosci* 2013; **14**: 365-76.

64. Song F, Eastwood AJ, Gilbody S, Duley L, Sutton AJ. Publication and related biases. *Health Technol Assess* 2000; **4**: 1-115.

65. Dwan K, Gamble C, Williamson PR, Kirkham JJ, Reporting Bias G. Systematic review of the empirical evidence of study publication bias and outcome reporting bias - an updated review. *PLoS One* 2013; **8**: e66844.

66. Guillen U, DeMauro S, Ma L, et al. Relationship between attrition and neurodevelopmental impairment rates in extremely preterm infants at 18 to 24 months: a systematic review. *Arch Pediatr Adolesc Med* 2012; **166**: 178-84.

67. Skiold B, Eriksson C, Eliasson AC, Aden U, Vollmer B. General movements and magnetic resonance imaging in the prediction of neuromotor outcome in children born extremely preterm. *Early Hum Dev* 2013; **89**: 467-72.

68. Setanen S, Lahti K, Lehtonen L, et al. Neurological examination combined with brain MRI or cranial US improves prediction of neurological outcome in preterm infants. *Early Hum Dev* 2014; **90**: 851-6.

69. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *J Pediatr* 1978; **92**: 529-34.

2.3 Summary of Chapter 2

This systematic review comprehensively evaluated the current literature on the ability of early MRI to determine motor outcomes in infants born preterm. Thirty articles were evaluated, 25 of which presented data of structural MRI and 16 of which presented diffusion MRI. The evidence suggests that early structural MRI has reasonable sensitivity and specificity to determine adverse motor outcomes and CP, while evidence for diffusion MRI is emerging. At this stage, structural MRI, evaluated qualitatively for evidence of injury or growth impairment is clinically accessible, while diffusion MRI and analysis remains restricted to research facilities.

Further evidence is required for early MRI, in particular with regards to diffusion imaging, which offers promise to further our understanding of preterm infant brain microstructural development. This motivated the design and development of the PPREMO study. A prospective cohort study design is the most robust study design methodology for observational studies, and so was the study design selected. The study aimed to recruit an unselected, consecutive cohort to ensure a representative sample of infants born <31 weeks GA. A number of studies in the systematic review performed a second MRI at TEA, and evaluated differences in measures between the early and term MRI in relation to outcome. Therefore, the PPREMO study was designed with both an early and term MRI. For MRI biomarkers at early or term MRI to be of clinical use, relationships with concurrent clinical measures obtained at the same time points, and then follow up with validated tools was imperative.

As a result, the study was designed with an MRI at 30-32 weeks PMA (‘Early MRI’) and at 40-42 weeks PMA (‘Term MRI’) and concurrent clinical assessment of motor, neurological, neurobehavioural and visual performance. Follow up at 3 months CA included gross motor and visual assessment. Neurodevelopmental outcome and presence/absence of CP was determined at 12 months CA. The following chapter presents the study protocol.

Chapter 3: Study Protocol

3.1 Introduction to Chapter 3

Chapter 3 consists of the study protocol titled, “PPREMO: a prospective cohort study of preterm infant brain structure and function to predict neurodevelopmental outcome”. This protocol paper delivers a detailed literature review, rationale and methods for the broader PPREMO study, the study initiated and established by this doctoral student, and within which this thesis is embedded. This thesis addresses the first three primary aims specified in the protocol paper, documenting the relationships between structural and diffusion MRI and clinical measures of motor, neurological and neurobehavioural function at Early and Term MRI, and then examining the ability of these measures of brain structure and function to predict motor and neurodevelopmental outcomes to 12 months CA. The remaining primary and secondary aims involve EEG and assessment of nutritional intake and their relationships with MRI and/or clinical measures. These elements are part of the broader PPREMO study, the analysis and interpretation of which form the basis of other student projects, or post-doctoral work. While EEG and infant nutrition are detailed in the protocol paper introduction and methods, they are beyond the scope of this thesis.

3.2 Paper 2:

George JM, Boyd RN, Colditz PB, et al. PPREMO: a prospective cohort study of preterm infant brain structure and function to predict neurodevelopmental outcome. *BMC Pediatr* 2015; **15**: 123.

PPREMO: A prospective cohort study of preterm infant brain structure and function to predict neurodevelopmental outcome

George JM, Boyd RN, Colditz PB, Rose SE, Pannek K, Fripp J, Lingwood B, Lai M, Kong A, Ware RS, Coulthard A, Finn C, Bandaranayake S

Abstract

Background More than 50 percent of all infants born very preterm will experience significant motor and cognitive impairment. Provision of early intervention is dependent upon accurate, early identification of infants at risk of adverse outcomes. Magnetic resonance imaging at term equivalent age combined with General Movements assessment at 12 weeks corrected age is currently the most accurate method for early prediction of cerebral palsy at 12 months corrected age. To date no studies have compared the use of earlier magnetic resonance imaging combined with neuromotor and neurobehavioural assessments (at 30 weeks postmenstrual age) to predict later motor and neurodevelopmental outcomes including cerebral palsy (at 12-24 months corrected age). This study aims to investigate i) the relationship between earlier brain imaging and neuromotor/neurobehavioural assessments at 30 and 40 weeks postmenstrual age, and ii) their ability to predict motor and neurodevelopmental outcomes at 3 and 12 months corrected age.

Methods/Design This prospective cohort study will recruit 80 preterm infants born ≤30 week's gestation and a reference group of 20 healthy term born infants from the Royal Brisbane & Women's Hospital in Brisbane, Australia. Infants will undergo brain magnetic resonance imaging at approximately 30 and 40 weeks postmenstrual age to develop our understanding of very early brain structure at 30 weeks and maturation that occurs between 30 and 40 weeks postmenstrual age. A combination of neurological (Hammersmith Neonatal Neurologic Examination), neuromotor (General Movements, Test of Infant Motor Performance), neurobehavioural (NICU Network Neurobehavioural Scale, Premie-Neuro) and visual assessments will be performed at 30 and 40 weeks postmenstrual age to improve our understanding of the relationship between brain structure and function. These data will be compared to motor assessments at 12 weeks corrected age and motor and neurodevelopmental outcomes at 12 months corrected age (neurological assessment by paediatrician, Bayley scales of Infant and Toddler Development, Alberta Infant Motor Scale, Neurosensory Motor Developmental Assessment) to differentiate atypical development (including cerebral palsy and/or motor delay).

Discussion Earlier identification of those very preterm infants at risk of adverse neurodevelopmental and motor outcomes provides an additional period for intervention to optimise outcomes.

Infants born very preterm (≤32 weeks gestational age; GA) are at a high risk of experiencing significant motor difficulties with 10-15% developing cerebral palsy (CP)[1], a further 40-50% having minor motor and behavioural difficulties[2, 3] and 30-60% experiencing cognitive difficulties at school age[4]. At least 25% of infants follow a trajectory of typical development with no evident sequelae of their difficult neonatal course[5]. Interventions are becoming available which aim to improve outcomes for infants born very preterm, necessitating the development of tools which can firstly identify those infants at risk of adverse outcomes as early as possible, and secondly provide accurate quantitative measurement of changes that are the result of an intervention. Currently, brain Magnetic Resonance Imaging (MRI) at term equivalent age (TEA) combined with the General Movements assessment (GMs) at 3 months corrected age (CA), show the greatest predictive accuracy of motor and neurodevelopmental outcomes and CP at 1, 2 and 5 years CA[6-10].

In preterm infants imaged at TEA, structural MRI (T1 and T2 weighted images) analysed qualitatively for evidence of white and gray matter abnormalities predict motor and cognitive outcome[8, 11], motor distribution of CP[12, 13], severity of motor involvement in CP[14] and neurobehavioural development[15]. White matter injury has been identified as the predominant injury in the preterm infant brain, with lesions such as periventricular leukomalacia (PVL) and intra-ventricular haemorrhage (IVH) well described and linked to poorer outcomes and CP[8, 16]. More recently, recognition of the intercurrent and subsequent developmental disturbances in both white and gray matter as a result of the primary lesion, support the description of preterm brain injury as an 'encephalopathy of prematurity'[17]. Qualitative classification of gray and white matter macrostructure from structural MRI has improved prediction of outcomes, but the need for quantitative microstructural information has lead to investigation of diffusion MRI in this population[18, 19].

Diffusion MRI measures the random motion of water molecules, which is hindered and restricted by the presence of cell membranes, the cytoskeleton, and macromolecules in the brain[20]. A number of quantitative metrics can be obtained from diffusion MRI to characterise the tissue, including fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) derived using the diffusion tensor model (i.e. Diffusion Tensor Imaging, DTI)[21]. These measures of the degree of restriction of diffusion (FA) and speed of diffusion (MD) change during brain development due to increasing fibre organisation, membrane proliferation, and myelination[22]. Diffusion MRI also provides estimates for the direction of the underlying white matter tracts, and, using tractography, enables the delineation of those pathways as they course through the brain.

White matter damage of prematurity is associated with increased values of MD and decreased values of FA[22, 23]. A significant correlation exists between values of FA in the corticospinal tracts and

postmenstrual age (PMA)[24] and between MD and later motor impairment[25]. Higher MD values at term are associated with poorer neurodevelopmental outcomes at 2 years in preterm infants[26]. Diffusion MRI has been reported to be an independent predictor of psychomotor delay[25] and to predict CP with a sensitivity of 80% (95% Confidence Interval [CI] 28-100) and a specificity of 66% (95% CI 53-78)[25]. Associations between FA values and cognitive outcomes have been reported[27]. The use of MRI tractography to predict neurodevelopmental outcomes is not yet well established[28].

Potential limitations of diffusion imaging such as complex crossing fibre microstructure, reliability and reproducibility, are being addressed through novel diffusion MRI acquisition and analysis techniques[29]. Customized for preterm babies, they include novel pre-processing, the use of 60-direction High Angular Resolution Diffusion Imaging (HARDI), high b values and fibre orientation distribution analysis[30]. These deal with the identified need for greater accuracy of tractography and improved quantitative markers[31].

Imaging technology advances are now able to be coupled with earlier imaging, with the advent of MRI compatible incubators. Safety and feasibility have been established for MRI in the neonatal period after birth and before TEA, with the potential to provide further insights into this period of rapid brain development[32-37]. At the stage very preterm infants enter the extra-uterine environment, between the end of the second and beginning of the third trimesters, cortical neurogenesis and migration are complete, axonal and dendritic branching continue vigorously, and synaptogenesis is commencing[38, 39]. From this stage until TEA is reached, white matter increases by 5 times the original volume, cortical gray matter volume increases 4 times and cortical folding both commences and is essentially completed[15, 40]. Brain development is rapid, vulnerable to injury but also adaptive to environmental inputs that guide and consolidate developing brain connections in a process termed neuroplasticity[41].

An area of specific interest in early imaging is the cortical subplate[42]. This structure consists of neurons formed in deep gray matter neurogenic sites such as the thalamus, and arrive to lie below the cortical neurons that migrated earlier from the subventricular zone[43]. At 30 weeks gestation, the subplate reaches its peak thickness, many times thicker than the cortex, and by term has almost completely regressed[44]. This major wave of growth and death establishes the long range projections between the deep gray matter and the cortex, and the short- and long- range cortico-cortical connections that are fundamental to integration of motor and cognitive functions[45]. This information on brain structure and structural connectivity from earlier neuroimaging increases the potential of understanding the trajectory of structural brain development.

Electroencephalography (EEG) is a useful method of measuring cortical function for diagnosis and predicting later outcomes. Relationships between EEG and structural and functional connectivity have been shown throughout development in both adults and infants[46-50]. Electroencephalography signals represent cortical electrical activity measured on the scalp and can be collected non-invasively with relative ease and low cost. Electroencephalography has strong predictive capacity for outcome in the term infant with hypoxic ischaemic encephalopathy[51]. Increasing use in the preterm population, particularly in configurations using a limited number of electrodes, are evidenced with the first reports of its utility in predicting outcome[52, 53]. Multi-channel EEG, typically 10-20 channels in the newborn, is well established in clinical practice and provides information about normal and abnormal functionality of the developing brain[54]. Deeper insights are possible with further analysis of multichannel EEG[55-58]. The power and the frequency of oscillations in the cortex can be assessed using power spectral density analysis[59].

Electroencephalography is able to define the electrical activity of the neonatal brain structural network that is visualised in diffusion imaging[30, 60, 61]. The electrical activity of these networks is characterised by two alternating modes observed in the amplitudes of EEG signals: a mode associated with the self-organising, locally generated spontaneous electrical activity transients (SATs) and a mode representing the low-amplitude intervals between SATs[62, 63]. This bimodality gradually attenuates from mid gestation and activity becomes continous by term[63].

In parallel to neuroimaging and neurophysiological modalities, several clinical assessments of neuromotor, neurobehaviour and neurological function are proposed for use in the preterm period and early infancy[64]. Two systematic reviews on the clinimetric properties of such measures found Prechtl's General Movements Assessment to have the greatest predictive accuracy of an outcome of CP[64, 65]. This neuromotor assessment evaluates spontaneous infant movement from preterm birth until 5 months CA[66]. A systematic review examining the accuracy of tests to predict CP included a meta-analysis of GMs and reported a pooled sensitivity and specificity of 98% (95% CI 74-100%) and 91% (95% CI 83-93%) respectively[10]. It is important to note that GMs at 3 months CA also predict severity of CP[67], cognition[68], minor neurological dysfunction[69] and behavioral and psychiatric outcome[70].

Neurobehaviour refers to an infant's ability to self-regulate, orient, be responsive to stimuli and sustain attention[71]. Neurobehavioural assessment in the preterm period reveals changes between birth and TEA, and differences between preterm and term infants assessed at TEA[72, 73]. Poorer neurobehavioural performance at TEA is associated with white matter abmormality on structural MRI, a range of adverse perinatal variables and predicts neurodevelopmental outcomes and CP at 18 months CA[72, 74, 75].

Components of the NICU Neonatal Neurobehavioural Scale, namely a low handling score, low movement score and high lethargy score are significantly related to an outcome of CP[75].

Neurological examination of infants offers reasonable prediction of outcomes, with sensitivity and specificity increasing as the infant progresses from the preterm period, through TEA and into the first year of life[10, 76]. Prediction of CP and motor outcome in the preterm period is relatively poor due to the presence of early transient abnormal signs with later good outcomes causing false positives and the converse resulting in false negatives[10, 77]. When neurological examination is performed before term age in preterm infants, the sensitivity for an outcome of CP is 57-86% and specificity 45-83%[78, 79]. At term, neurological assessment has a sensitivity of 88% and specificity of 46% to predict structural MRI abnormalities[80] and 68-79% and 63-70% to predict CP[78, 81]. In the post term period sensitivity and specificity range from 68-96% and 52-97% respectively[78, 81].

Perinatal factors, including growth and nutrition, have been identified as risk factors of adverse outcomes. Poor growth during the first weeks after preterm birth is a significant predictor of poor neurodevelopmental outcome[82-84]. Increased nutrient intake leads to better growth[85-87], and presumably better brain development, although this relationship is not proven. There is a need for clear evidence of the relationship between early nutrient intake and brain development in preterm infants, so that improved nutrient regimens can be designed.

Individual modalities of MRI, EEG, clinical measures, perinatal risk factors and nutrition have been evaluated in relation to later outcomes for preterm infants as described above. Combinations of modalities have been evaluated and often demonstrate improved prediction of outcomes over individual modalities alone[6, 7, 10, 88, 89]. The relationships between modalities at TEA are emerging, but to our knowledge, few studies to date have examined the relationships between early clinical measures, perinatal risk factors and nutrition, and very early imaging at 30 weeks PMA[7, 15, 72, 73, 80, 90-92]. This study aims to contribute to the understanding of brain structure-function relationships in the very early phase of the developmental trajectory, improving the ability to identify infants at risk of adverse outcomes, facilitating innovation of interventions and developing quantitative biomarkers of brain development.

Broad aim: This prospective cohort study of infants born ≤30 weeks will investigate the relationship between brain structure (structural and diffusion MRI), brain function (neurological, neuromotor, neurobehaviour, vision and EEG), perinatal risk factors and nutrition of very preterm infants in the preterm period (30-32 weeks) and at TEA; then examine the ability of these early measures to predict motor and neurodevelopmental outcomes at 3 and 12 months CA.

Primary aims: In a prospective cohort study of infants born at ≤30 weeks, and a term reference group, this study aims:

1. To examine the relationship between brain structure on structural and diffusion MRI, brain function on clinical measures of neurological, neuromotor and neurobehavioural performance, and perinatal risk factors at 30 and 40 weeks PMA.
2. To determine whether brain structure and function at 30 weeks PMA predicts outcomes of brain structure and function at 40 weeks PMA, 3 months CA and 12 months CA.
3. To evaluate the ability of structural and diffusion MRI and functional measures at 30 and 40 weeks PMA age to predict motor outcome at 3 months CA and motor, neurodevelopmental outcome and CP at 12 months CA.
4. To evaluate the ability of perinatal variables and social risk (socio-economic status; SES) to predict severity of motor outcome and CP at 12 months CA.

Secondary Aims:

1. To examine the development of motor, sensory, visual and auditory connectivity between 30 week and 40 week MRIs in infants born preterm with and without brain lesions.
2. To examine the correlation between brain function on dense array EEG, and motor and visual outcomes at 40 weeks PMA.
3. To evaluate the ability of dense array EEG at 40 weeks PMA to predict visual outcome at 3 months CA and cognitive outcome at 12 months CA.
4. To examine the correlation between data fusion of brain functions on dense array EEG and brain structure on diffusion MRI, and motor and visual outcomes at 40 weeks PMA.
5. To evaluate the ability of data fusion of brain functions on dense array EEG and brain structure on diffusion MRI, to predict visual outcome at 3 months CA and cognitive outcome at 12 months CA.
6. To examine the relationship between preterm macronutrient intake from birth to 34 weeks and brain development at 40 weeks post menstrual age, and determine if nutritional intake is more predictive of brain development than other maternal and neonatal risk factors.

Hypotheses

The specific hypotheses to be tested include the following. In infants born very preterm:

1. A strong correlation exists between MRI, clinical measures and perinatal variables at 30 weeks PMA.
2. Brain structure and function at 30 weeks PMA predicts outcomes at 40 weeks PMA, 3 months CA and 12 months CA.

3. Brain structure and function at 40 weeks PMA predicts neurodevelopmental outcome at 3 and 12 months CA.
4. A strong correlation exists between EEG, clinical measures and perinatal variables at 40 weeks PMA, and 3 months and 12 months CA.

Methods and analyses

Design

A prospective observational cohort study of infants born very preterm with a comparison group of infants born at term.

Ethical considerations

Ethical permission to conduct the study has been obtained from the Human Research Ethics Committees at The Royal Brisbane & Women's Hospital (HREC/12/QRBW/245), and The University of Queensland (2012001060). The trial has been registered with the Australian New Zealand Clinical Trials Registry (ACTRN12613000280707). Participation in the study is voluntary, written informed consent for participation in the study is obtained from a parent or guardian, and families may withdraw from the study at any time without explanation.

Study sample and recruitment

Preterm sample

This study aims to recruit 80 preterm infants from the Neonatal Intensive Care Unit (NICU) at the Royal Brisbane and Women's Hospital (RBWH). A research nurse will screen infant admissions for eligibility, and determine the appropriate stage to approach the family based on medical stability and approval from the treating neonatologist. Eligible families will be approached and if they express an interest in the study, they will be provided with detailed information and an explanation of the study. Parents will be given the opportunity to ask questions and discuss involvement with their treating clinician prior to making their decision. Informed written consent will be obtained from parents or guardians interested in participating and their infant will be formally enrolled.

Inclusion criteria

Infants born at ≤30 week's gestation, who live within 200km of the hospital to allow for follow up hospital appointments and home visits, and have English speaking families as there is insufficient funding for translators, are eligible for this study.

Exclusion criteria

Infants diagnosed with any congenital or chromosomal abnormality that could adversely impact neurodevelopmental outcome, and/or any contraindications to MRI, are ineligible for this study.

Term reference sample

Twenty term born babies will be recruited from either the postnatal ward of the RBWH, or as interested volunteers by word of mouth.

Eligibility criteria

Infants are eligible to participate in the reference sample if they are born between 38 and 41 weeks gestation following an uncomplicated pregnancy and delivery, have a birth weight above the 10th percentile, and are not admitted to neonatal intensive or special care units following their birth.

Sample Size

There are no data currently available to assess the relationship between MRI and clinical measures at 30 weeks PMA to predict motor outcome at 3 months CA and motor/neurodevelopmental outcome or CP at 12 months CA. Sample size calculations are based on a study investigating the ability of MRI at TEA, and the GMs assessment, to predict motor outcomes and CP at 12 months CA [6]. In a prospective cohort of infants born <30 weeks GA and in a total sample size of n=86, MRI was classified as normal (n=22), or with mild (n=54), moderate (n=6) or severe (n=4) white matter abnormality (WMA) [93]. Infants with normal or mild WMA were grouped (n=76), and infants with moderate and severe WMA were grouped (n=10) [6]. We assume the same ratio (7.6 MRI normal or with mild/moderate WMA: 1 MRI with moderate/severe WMA) will be observed in this study. Of the n=10 infants in the prior study that had moderate/severe WMA, n=5 (50%) developed CP [6]. If we assume that 5% of infants with MRI normal or with mild/moderate WMA develop CP, then the study requires 69 infants to be recruited (8 with MRI with moderate/severe WMA and 61 with MRI normal or with mild/moderate WMA) in order to be able to reject the null hypothesis that the proportion of infants with CP in the two groups are equal with power=90%. The Type I error probability associated with this test of this null hypothesis is 0.05. In order to explore WMA earlier, at 30 weeks PMA, and its ability to predict CP at 12 months CA, an increase in the projected numbers will be required, and a further 15-20% added to account for attrition. Consequently, the aim is to recruit a total sample size of 80 infants with full data sets.

Perinatal data collection

An extensive record of the pregnancy, birth history, and neonatal course will be collected from the medical discharge summary. This will allow detailed description of the characteristics of the sample, allow comparison to outcomes establishing predictor variables, and to adjust for confounders.

A number of prenatal variables have been shown to impact short and long-term outcomes. Prolonged rupture of membranes, defined as spontaneous rupture of membranes ≥24 hours before

delivery is the most significant risk factor of a poor outcome among pregnancy history[94, 95]. Maternal antenatal corticosteroid administration reduces the risk of neonatal death and respiratory distress (complete course defined as more than 1 dose of steroids given, and 1st dose at more than 24 hours and less than 8 days before birth)[94-96]. Evidence also exists for antenatal steroids protecting against cerebral haemorrhage[97]. The neuroprotective effect of magnesium sulphate administration reduces the risk of an outcome of CP (relative risk 0.68, 95% confidence interval 0.54 to 0.87)[98]. Assisted conception is associated with adverse neurodevelopmental outcomes independent of prematurity, multiple pregnancy and gender for infants born between 22-26 weeks gestation[99]. Multiple birth status will be examined as the widely held view that singletons experience better outcomes than multiples has recently been challenged. In a population based study of n=1473 born <29 weeks gestation, infants from multiple gestation pregnancies demonstrated comparable neurodevelopmental outcomes to singletons[100].

Birth history variables collected will include GA at birth, gender and birthweight. The risk of CP and adverse neurodevelopmental outcomes increases with decreasing GA at birth[101], and multiple studies report poorer outcomes for male infants[94, 102-104]. Intra uterine growth retardation (IUGR) can result in decreased cortical volume, poorer outcomes and increased risk of neonatal complications[105, 106], and babies that are small for gestational age (SGA) are at a higher risk of death, adverse neonatal outcomes and neurodevelopmental impairment[107]. Growth restriction in this study will be defined as a birth weight <10th percentile based on the Olsen growth curves.

Information will be gathered over each infant's neonatal course from birth until discharge from hospital. Cranial ultrasound findings, specifically findings of PVL and IVH graded according to the criteria of Papile et al, 1978 will be documented, with higher grades predictive of adverse outcomes and CP[108]. Necrotising enterocolitis (NEC) is associated with poorer growth, cognitive and motor outcomes, and is considered proven if the infant warranted treatment which included nil by mouth and antibiotics[95, 109]. Late onset sepsis is a significant risk factor, diagnosed by isolation of an organism from at least one blood culture and a decision to give antibiotics with therapeutic intent, from 48hrs after birth[94, 95]. Culture proven sepsis is independently associated with an outcome of CP[110]. Postnatal corticosteroid use demonstrates an independent effect on poor outcome, in particular with behavioural outcomes and CP[94, 111, 112]. Bronchopulmonary dysplasia or chronic neonatal lung disease are independent risk factors for adverse neurodevelopmental outcomes due to recurrent episodes of hypoxia[111, 113-117]. Chronic neonatal lung disease is defined as babies born <32 weeks GA requiring any respiratory support or supplemental oxygen for a chronic pulmonary disorder at 36 weeks PMA[95]. Postmenstrual age at NICU discharge will be documented, as poorer behavioural outcomes are associated with longer length of hospital stay[118].

For each infant from birth until 34 weeks PMA, the daily intake of all nutrient-containing solutions will be recorded. Intake of protein, lipid, carbohydrate and energy for each day will be calculated by multiplying intake volumes for each solution administered by the nutrient concentration obtained from manufacturer's specifications or, for breast milk, published data[119].

Socio-demographic information such as maternal and paternal education and occupation will be collected using a baseline parent questionnaire (see Additional file 1). Social and environmental factors may impact infant development, and low socio-economic status and parenting factors have been shown to adversely influence outcomes[120]. Social risk will be assessed using a score measuring six aspects of social status including: family structure, education of primary caregiver, occupation of primary income earner, employment status of primary income earner, language spoken at home and maternal age[116, 121, 122]. Each item will be scored between 0 and 2 for a total score of 12, with scores of 2 and above being considered high social risk in line with other research in this population[121, 122]. Higher social risk has been strongly associated with later behaviour problems, and independently predicts a lack of early intervention services[122, 123]. A recent systematic review found evidence that lower socio-economic status results in an additional risk of CP, over and above the risks conferred by prematurity or lower birthweight[124].

Procedures

Study procedures are depicted in Figure 1. Participants will be recruited, consented and enrolled as described above. Between 30-32 weeks PMA, when medically stable, infants will undergo an MRI. In the event an MRI cannot be undertaken due to medical instability, MRI's will be conducted when the infant becomes medically stable and up to a maximum age of 36 weeks PMA. This will ensure that less fragile infants are not over-represented in the sample. The following day, infants will undergo clinical assessment by an assessor blinded to GA at birth, CUS and MRI findings and any unrelated medical information, and a video recording of their spontaneous movements will be captured. As there is no established gold standard neurological or neurobehavioural assessments for use at this time point, a combination of the NICU Neonatal Neurobehavioural Scale (NNNS), Hammersmith Neonatal Neurological Examination (HNNE), and the Premie-Neuro will be used[125]. These assessments will be combined to minimise handling and modified to remove items unsuitable for administration at this age. The assessment time will be 10-15 minutes, conducted before a scheduled feed and cares to ensure optimum comfort and alertness. Infant cues, physiological signs of stress or distress, oxygen saturations and heart rate will be monitored throughout, and the assessment paused or discontinued where necessary. The assessment will be video recorded for independent scoring and testing of inter- and intra-rater reliability.

At TEA the family will be invited to return for their infant to undergo a second MRI and an EEG. The following day an assessor blinded to GA at birth and CUS and MRI findings will visit the family at home to undertake the clinical assessments. A video of the infant's spontaneous movement will be recorded for later scoring of the GMs assessment, a brief assessment of visual function will be undertaken and 3 motor and neurobehavioural assessments will be administered, combined to remove duplicate items. The NNNS assessment, which is highly structured, will be completed first, followed by the few additional items of the HNNE and the Test of Infant Motor Performance (TIMP). Total assessment time will be approximately 1 hour, however, the assessment will be conducted at the infant's pace, and breaks for feeds or sleep will be undertaken as appropriate.

At 3 months CA, during a home visit, a GMs video of the infant's spontaneous movement will be taken, and a visual assessment and the TIMP will be completed. The total assessment time will be approximately 40 minutes.

At 12 months CA, families will be invited back to the RBWH for follow up assessment of their child's motor and neurodevelopmental outcome. In a telephone call prior to the appointment a research nurse will gather up to date information on the child's current medical team, medical history since discharge, any diagnoses made and details of any interventions they have received. A paediatrician blinded to medical history will assess for signs of neurological abnormality and the presence of features of CP. A physiotherapist blinded to background history will conduct neurodevelopmental and motor assessments. As no single measure has been shown to provide conclusive data on attainment and quality of motor skills in this population, a combination of the Bayley Scales of Infant and Toddler Development III (Bayley III), the Neurosensory Motor Developmental Assessment (NSMDA) and the Alberta Infant Motor Scale (AIMS) will be performed[126]. The total assessment time will be 1-1.5 hours.

Measures

MRI Methods

Image Acquisition: Brain MRI will be performed using a 3T (Siemens Tim Trio, Erlangen, Germany) and an MR compatible incubator with dedicated neonatal head coil (LMT Lammers Medical Technology, Lubeck, Germany). Noise from the MRI will be attenuated using Natus Mini Muffs (Natus Medical Inc., San Carlos, CA). The preterm group will have an MRI at 30-32 and again at 40-42 weeks PMA. The term group will have an MRI at 40-42 weeks PMA. All infants will be monitored with pulse oximetry and electrocardiographic monitoring. Infants will be fed, fitted with ear protection to minimize noise exposure, carefully wrapped and placed in the incubator

in the scanner without sedation or anaesthesia. The total scanning duration will be approximately 45-60 minutes for each baby. Where possible, images impacted by significant motion artefacts will be rescanned. The MR protocol will include T1, T2 TSE, T1w MPRage, T2w HASTE and 3 echo T2 map, Arterial Spin Labelling (ASL), 30 direction diffusion weighted imaging (DWI), and 64 direction DWI sequences. Additional file 2 outlines the MRI protocol parameters. A neuroradiologist will review clinical sequences and classify white and gray matter injury[93, 127].

Quantitative T2 will be measured using a T2 image series acquired with echo times of 27, 122 and 189 ms and repetition time 10580 ms; 47 axial contiguous slices of 2.0 mm thickness will be acquired with a 144 × 180 mm field of view, a flip angle of 150°, and a 153 × 256 matrix (reconstructed to 204 × 256), resulting in voxel sizes of 0.70 × 0.70 x 2.0 mm^3. T1-weighted magnetization prepared rapid-acquisition gradient echo volumes in the sagittal plane will be acquired with an echo time of 3.21 ms and repetition time 2100 ms; 96 sagittal slices of 1.3 mm thickness will be acquired with a 160 mm field of view, a flip angle of 9°, and a 128 × 128 matrix, resulting in voxel sizes of 1.25 × 1.25 × 1.3 mm^3.

Diffusion images will be acquired using single-shot echo planar multi-direction diffusion-weighted sequence, employing dual bipolar diffusion gradient and double spin. This will include the acquisition of a 30 direction DWI protocol (b=1000 s/mm^2) and a 64 direction HARDI protocol (b = 2000 s/mm^2). The images will be acquired per location, consisting of one low (b=0s/mm^2) and the rest high (b=1000 or 2000s/mm^2) diffusion-weighted images, in which the encoding gradients are uniformly distributed in space. Imaging parameters of the diffusion sequence will be: field of view 224 x 224 mm, matrix 128×128, repetition time 9500 ms, echo time 130 ms and flip angle of 90°. A field map for diffusion data is acquired using two 2D gradient recalled echo images (TE1/TE2 4.9/7.4ms) to assist in correction for residual distortions due to susceptibility inhomogeneity's (acquisition time 1m). These sequences allow exploration of brain microstructure and function, specifically: (i) regional and global cortical surface and thickness, (ii) white matter organisation, (iii) structural connectivity of relevant areas and (iv) pre-myelination (T2).

Arterial spin labelling MRI provides a non-invasive technique to measure cerebral blood flow (CBF), although its feasibility and value in neonates is largely unknown. As the neonate's brain rapidly grows, it is anticipated that an associated increase in CBF would occur to supply the nutrients and energy needed for the added brain weight[128]. Arterial spin labelling MRI will be performed using a PICORE Q2TIPS sequence with echo-planar imaging. Imaging parameters of the ASL scan will be: field of view 256 mm, matrix 64×64, repetition time 3427.5 ms, echo time 21 ms, inversion time of arterial spins (TI1) 700 ms, saturation stop time 1600 ms, total transit time of the spins (TI2) 1800

ms, tag thickness 100 mm, tag to proximal slice gap 25 mm, 17 axial slices, slice thickness 5 mm, time lag between slices 22.5 ms, and Bandwidth Per Pixel Phase Encoding time of 23.343 ms.

Image Analysis: MRI data will be analyzed using advanced image processing techniques as below.

a) Structural Analysis:

T2 relaxation maps will be obtained from three T2-weighted images by first aligning all T2-weighted images to the T2-weighted image with the shortest echo time (TE=27ms) using rigid-body registration, followed by voxel-wise estimation of T2 employing a nonlinear least-squares fit. The T2w MR will be segmented using the MILXView neuroimaging platform with the UNC neonate atlas and ALBERT atlas used to provide initial priors and anatomical labelling[129-131]. Statistical analysis will use Regions-of-Interest and voxel based analysis techniques. Summary measures of T2 will be calculated within pathways delineated using tractography.

b) Diffusion Analysis:

An extensive pre-processing and quality control procedure will be used to detect and correct image artefacts caused by involuntary head movement, cardiac pulsation, and image distortions[30]. Fractional anisotropy (FA) and mean diffusivity (MD) will be estimated from corrected diffusion data using a diffusion tensor model. Constrained spherical deconvolution implemented in MRtrix will be employed to estimate fibre orientation distribution (FOD)[132]. Whole-brain voxel based analysis of FA and MD will be performed using tract-based spatial statistics optimised for neonates[133]. Whole-brain voxel-based analysis of fibre orientation distributions will be conducted using Apparent Fibre Density (AFD)[31]. Probabilistic tractography will be performed using MRtrix. White matter pathways will be delineated using the multi-regions-of-interest approach. A number of pathways, including cortico-spinal tract, corpus callosum, superior longitudinal fasciculus and thalamic radiations, will be extracted. Summary measures of FA, MD, AFD and T2 within pathways will be calculated.

c) Arterial Spin Labelling analysis:

An extensive pre-processing and quality control procedure will be used to detect and correct image artefacts caused by motion, random thermal and physiological noise, EPI distortion, spatial-temporal denoising, correction for temporal decay and partial voluming of the signal. The CBF maps will then be calculated in absolute units ($\frac{ml}{100g\ 60s}$), with the first equilibrium magnetization of arterial blood estimated using the calibration image (first acquired image), and GM and WM maps rescaled. Statistical analysis will use Regions-of-Interest and voxel based analysis techniques.

EEG

Dense array EEG (dEEG) will be collected using either; i) a NicOne EEG amplifier (Cardinal Healthcare, USA) with a sampling rate of 256 Hz from 32 channels using an appropriately sized EEG cap (Waveguard, ANT-Neuro, Germany) with electrode positioning according to the international 10-20 standard, or ii) a 64-electrode high-density sensor net (HydroCel Geodesic Sensor Net, Electrical Geodesics Inc.). Each electrode is enclosed in a saline sponge, in a geodesic tension structure comprised of elastic threads. EEG signals are transmitted from the sensor net electrodes to an amplifier (Electrical Geodesics Inc.) digitised and recorded via NetStation software (Electrical Geodesics Inc.).

For the EEG data i) directional relationships between channels, ii) frequency-specific amplitude fluctuations, and iii) time-varying behaviour through directional connectivity analysis and phase synchrony among channels will be examined. Electroencephalography power will be estimated in the frequency bands delta/theta (2–7 Hz), alpha (8–13 Hz), beta (14–32 Hz) to examine changes in the power and frequency of oscillations over the sensorimotor cortex as an index of corticospinal linkage and maturation[59].

The electric resting state network (eRSN) analysis will follow a multi-step procedure comprising i) pre-processing of EEG signals, ii) extracting band amplitude fluctuation envelopes at the frequency band of interest, and iii) evaluating their network characteristics within two modes of activity. Relationships between eRSN characteristics and outcome will be sought using approaches including pair-wise relationships such as mutual information measures, with testing using surrogate signals as well as different statistical testings at individual and group levels.

Clinical measures

General Movements Assessment (GMs): The GMs is a predictive and discriminative tool that involves observation of an infant's spontaneous motor activity[66]. It can be used from preterm birth until 20 weeks CA and is carried out by videoing the infant in supine, in a calm alert state with no external stimulation. Scoring is completed from the recording with 3 full movement sequences required for pattern recognition (approximately 5 minutes)[66]. In the early preterm stage this may require up to an hour of video in order to select sequences of active movement, but at TEA and 12 weeks CA it may only take a few minutes. Movements are classified as normal or abnormal (poor repertoire, cramped synchronised or chaotic) in the writhing period from preterm up to 6 weeks post term. During the fidgety period from 9-20 weeks post term, fidgety movements are classified as present, abnormal or absent[66]. Infants in this study will have an assessment of their GMs in the preterm period (30 - 32 weeks PMA), one assessment at TEA, and one at 10-12 weeks CA. The

GMs have been found to have the greatest predictive accuracy of motor outcome in two systematic reviews on the clinimetric properties of neuromotor and neurobehavioural assessment tools for use in preterm infants in the preterm period and first year of life[64, 65]. A systematic review examining the accuracy of tests to predict cerebral palsy included a meta-analysis of GMs. The pooled sensitivity and specificity were 98% (95% CI 73-100%) and 91% (95% CI 83-95%) respectively[10]. General Movements in the fidgety period display greater sensitivity and specificity than those in the writhing period[6, 7, 134] and have also shown an ability to predict functional severity of CP as classified by GMFCS[67]. Additionally, GMs predict cognition[68, 135, 136], minor neurological dysfunction and developmental coordination disorder[69, 137], as well as behavioral and psychiatric outcomes[70, 138].

The NICU Network Neurobehavioural Scale (NNNS): The NNNS is a discriminative neurobehavioural assessment initially designed for use in prenatally substance exposed infants as part of the Maternal Lifestyle Study (MLS)[139]. Its application for use in other high-risk infant populations including very preterm infants is now well established[64, 75, 125]. Neurobehavioural functioning is determined through evaluation of neurological and motor performance, orientation to auditory and visual stimuli, state regulation, self-soothing competence and stress signs. Forty-five items are administrated in a structured format comprising state-dependent 'packages', with a further 21 summary items scored. The stress/abstinence scale encompasses an additional 51 observed items. Summary scores are calculated to enable statistical analysis, and they include orientation, habituation, hypertonicity, hypotonicity, excitability, arousal, lethargy, nonoptimal reflexes, asymmetric reflexes, stress, self-regulation quality of movement and handling[140]. Training and certification is required to administer and score the assessment.

Normative data on the NNNS are available in 2 studies, with samples of 125 and 344 healthy term infants respectively, assessed within 48 hours of birth[141, 142]. Data of preterm infants assessed using the NNNS at 1 month CA are available though it is important to note that the cohort is selected from the MLS sample and therefore includes infants with high social risk and drug-exposure[143]. Preterm infants display poorer neurobehaviour at TEA when compared to term controls on the NNNS[73, 144]. Significant disturbances were found in motor behaviour, tone, poorer self-regulation capacities, higher excitability scores[144], poorer orientation, lower tolerance of handling and more stress in preterm infants compared with term born infants[73]. These alterations in neurobehaviour correlated with cerebral abnormalities in white and gray matter on qualitative structural MRI[72]. Predictive validity of the NNNS has been established with neurobehaviour at term predicting motor and cognitive outcomes at 18 months, motor outcomes at 24 months and cognitive outcomes at 4.5

years[75, 145, 146]. Test-retest reliability has been established with preterm infants with correlations ranging from .30 to .44 across three time points tested (34, 40 and 44 weeks PMA)[147].

Hammersmith Neonatal Neurological Examination (HNNE): The HNNE was developed for the assessment of term and preterm infants at risk of developmental delay[148-150]. It is a discriminative and predictive test that assesses posture and tone, reflexes, movements and neurobehavioural responses. It is criterion and norm referenced, with normative data from a sample of 224 healthy low-risk term infants assessed between 6 and 48 hours after birth[149]. Raw scores are converted into a continuous score derived through optimality scoring with final scores ranging between 0-34, and scores <30.5 considered to be suboptimal[150]. Preterm infants have been found to have poorer scores on the HNNE compared with term born infants when assessed at TEA. In a sample of 157 infants born at <33 weeks GA mean optimality scores were 26.4[151]. Discriminative validity was demonstrated in a normative study of a sample of 380 preterm infants (GA at birth 25-35 weeks) with a normal outcome and a sample of 85 infants who developed CP examined at TEA. Preterm infants with later outcome of CP had a greater number of suboptimal items scored compared to those preterm infants who had a normal outcome[152]. Concurrent validity has been demonstrated in 2 studies (n=168 and n=66), where poorer scores on the HNNE related to increasing severity of cerebral abnormality on structural MRI[72, 80]. A systematic review examining the predictive validity of the HNNE to predict an outcome of CP report a sensitivity range of 57-86% and specificity range of 45-83% when performed before term age (<37 weeks PMA)[78, 79]. This increases to a sensitivity range of 68-96% and specificity range of 52-97% when assessed in the post term period[78, 81]. Percentage agreement has been shown to be good between raters after training (>96%)[153], however few reliability statistics are available. The infants in the present study will have the HNNE assessment at 30 weeks gestation, and TEA.

Premie-Neuro: The Premie-Neuro is a neurological and neurobehavioural assessment tool developed by Ellison and Daily[154]. It consists of 3 subscales of 8 items each: neurologic, movement and responsiveness. Although limited published data are available for this relatively new tool, it was selected for this study for the following reasons: i) scoring of neurologic and movement subscales can be completed in even the sickest and most fragile of infants as they require minimal handling, ii) significant overlap with the HNNE and NNNS means the assessment can be scored with the addition of only 2 items overall, iii) scores are based on expected findings at differing gestational age[154]. Validity has been established for discriminating between preterm infants at high and low risk for neurodevelopmental delay, although interrater reliability was low and test–retest reliability was fair to moderate[155]. It will be scored from the combined assessment performed at 30 weeks PMA for infants in this study.

Neonatal Visual Assessment: The neonatal assessment of visual functions provides useful information on various aspects of early neonatal visual function, including ocular motility, fixation, following, acuity and attention at distance. The battery is easy to perform, does not require long training, and can be performed reliably from 32 weeks PMA[156]. It has been demonstrated to contribute to prediction of neurodevelopmental outcome in preterm babies[157-159]. The overall sensitivity and specificity of Neonatal Visual Assessment to predict 12 month CA visual scores were 90% and 63% respectively in 121 preterm infants[158]. In this study, infants will be assessed at TEA and 12 weeks CA.

Test of Infant Motor Performance (TIMP): The TIMP is a discriminative and evaluative test of functional motor behaviour used to assess infants between the ages of 34 weeks PMA and 4 months CA[160, 161]. The test assesses the postural and selective control of movement needed for functional motor performance in early infancy and is norm referenced. Observational and elicited items are administered in a standardised procedure and the test takes 20-40 minutes to administer. At 12 weeks CA, the TIMP has been shown to predict 12 month motor performance with sensitivity 92% and specificity 76%[162] and preschool motor performance (mean age 4.75 years) with sensitivity 72% and specificity 91%[163]. In this study, the TIMP will be performed at TEA and at 12 weeks CA by an assessor trained by the test author.

Neurodevelopmental and motor outcome at 12 months

Medical Assessment: A paediatrician experienced in infant development and diagnosis of CP will independently assess infants in this study at 12 months CA. The purpose of this assessment is to discriminate which infants are developing typically from those who are not, and to confirm diagnoses of CP or not CP[164]. It is acknowledged that 12 months CA is early to confirm a diagnosis of CP, especially in less severe cases. For this reason a structured neurological examination of posture, reflexes, muscle tone and movement will be conducted with participants classified as 'normal' (entirely normal neurological examination), 'unspecified signs' (e.g. hypotonia, asymmetric reflexes) or 'abnormal' (definite neurological abnormality, likely CP). In cases where CP can be confirmed, motor type and distribution will be recorded as per the SCPE guidelines[165], and functional severity established through classification with the Gross Motor Function Classification System (GMFCS)[166]. The assessment will be videoed and a second blinded assessor will perform this classification for reliability purposes.

Bayley Scales of Infant and Toddler Development III (Bayley III): The Bayley III is a discriminative tool designed to assess cognitive, language and motor development, and social-emotional and adaptive behaviour[167]. It is currently the most widely used assessment tool for overall neurodevelopment in follow up

studies of preterm infants between 1 and 3 years CA. It is a norm-referenced test with normative data for the cognitive, language and motor subscales taken from a sample of 1700 American infants and children[167]. Normative data for the adaptive behaviour scale was obtained independently in a sample of 1350 infants and children[167]. Normed scores of the Bayley III have a mean of 100 and a SD of 15, where higher scores reflect better development. Bayley III Motor Composite score correlates with the second edition of the Peabody Developmental Motor Skills (r =0.57)[167]. Reliability has been established with the average reliability coefficients for the composite scale scores range from .91 (Cognitive) to .93 (Language)[167]. In a systematic review of the predictive value of the Bayley III on development of very preterm infants, mental development index scores were strongly predictive of later cognitive functioning (14 studies with a total sample n=1330 children), r=0.61 (95% CI: 0.57-0.64)[168]. Motor scale scores were only moderately predictive of later motor function (across 5 studies with a total sample of n=555 children), r=0.34 (95%CI: 0.26-0.42). For this reason, a further two assessments which are primarily motor assessments, and have stronger psychometric properties will be used, the NSMDA and the AIMS[65]. The Bayley III involves interaction between the infant and the examiner in a standardised series of play tasks, and takes 45-60 minutes to administer at 12 months CA.

Neurosensory Motor Developmental Assessment (NSMDA): The NSMDA is a discriminative and predictive, criterion-referenced test of gross and fine motor development[65, 169]. It examines gross and fine motor performance, neurological status, posture, balance and response to sensory input. The examiner observes and administers items and the test takes 10-30 minutes to complete. The results give a total score and a functional classification of motor development as normal, or with mild, moderate or severe problems of posture, movement and co-ordination. Assessment at 4 months predicts outcomes at 24 months with a sensitivity of 80% and a specificity of 56%[170.] Studies looking at the longer term predictive validity of the NSMDA, found assessment at 12 months had strong associations with motor and cognitive scores at 4 years[171], and NSMDA assessment at 8 months to have an 80% sensitivity of motor outcomes at 11-13 years in extremely low birth weight infants with no apparent neurological deficit or CP[172]. The NSDMA will be used to classify each infant's development as normal or as having mild, moderate, severe or profound motor dysfunction at 12 months CA.

Alberta Infant Motor Scale (AIMS): The AIMS is a discriminative, norm-referenced tool that tests gross motor skills through the components of weight bearing, posture and antigravity movements[162, 173]. The test involves observation of the infant in prone, supine, sitting and standing and is able to be completed in this study purely through observation during the Bayley III and NSMDA assessments with no additional handling. Normative data are based on a population of 2200 term infants from 0-18 months in Alberta, Canada[174], and when recently compared with a contemporary sample of 650 Canadian infants, found to still be relevant. Normative data for preterm infants has also been published with a sample of 800 infants born at $\leq$32 weeks from the Netherlands[175]. Raw scores are

obtained with centile ranks and age equivalent growth scores available for term and preterm infants. The AIMS has high inter-rater reliability (ICC= .98 to .99)[176, 177], and intra-rater reliability (ICC= .97-.99)[177]. Concurrent validity with the Bayley II at 12 months CA in a cohort of preterm infants has been established (r= .90)[177]. Although the AIMS was not designed as a predictive tool, it has moderate to excellent predictive validity. In a sample of 164 preterm infants assessed at 8 months CA, the AIMS predicted motor outcomes at 18 months CA with a sensitivity 86.4% and specificity 93%[178]. The suitability of using the AIMS as a discriminative and predictive tool at 12 months CA in preterm infants has been supported by a clinimetric review of neuromotor measures for preterm infants in the first year of life[65]. The AIMS will be used to classify each infant's development as normal or suspicious/abnormal at 12 months CA in this study.

Blinding

The researchers involved in MRI and EEG analysis (KP, JF, SER, MML, and AHTK) will be blinded to GA at birth, CUS findings and clinical assessment findings. The researchers carrying out the clinical assessments and scoring (JMG, PBC) will be blinded to gestational age at birth, MRI and CUS findings. Outcome assessments at 12 months CA will be performed and scored by assessors blinded to infant perinatal history, MRI and early clinical assessment findings.

Adverse events

There are no known health or safety risks related to any aspect of the described study. There are no known risks for MRI and no sedation will be used. The principal researchers RNB, PBC and SER will review any adverse event or unintended effect detected.

Data analysis and statistical considerations

When models involve brain structure and function data from one time point (either 30-32 or 40-42 weeks), standard regression models will be constructed; when models use data from both 30-32 and 40-42 weeks, mixed-effects models that take into account within-infant correlation will be used. Models will be constructed using standard principles; first univariable analyses will be used to identify variables significant at the $p<0.15$ level and these variables then entered into multivariable models one-by–one, in decreasing order of significance. At each step the current model will be compared to previous models using the likelihood ratio test. Linear regression will be used for continuous outcomes (e.g. diffusion MRI measures of FA and MD); logistic regression for binary outcomes (e.g. disability/no disability); and multinomial logistic regression for categorical outcomes with > 2 categories (e.g. NSMDA categories of normal/suspect/abnormal). Results will be presented as effect estimates and 95% confidence intervals. The sensitivity and specificity of the predictive assessment model will be determined based on diagnosis of disability using standard

definitions. Perinatal, clinical, demographic and social characteristics will be included as covariables when appropriate. Analyses will be supervised by RSW, a senior biostatistician at The University of Queensland.

Discussion

To our knowledge, this protocol describes the first study examining the clinical correlates of early advanced brain imaging and clinical measures at 30 weeks PMA to predict motor and neurodevelopmental outcomes at 3 and 12 months CA. The results of this study will i) establish the relationships between early clinical measures, EEG, perinatal variables and nutrition and early advanced neuroimaging at 30 weeks PMA, ii) establish which components of brain structure and function most accurately predict neurodevelopmental, motor outcomes and CP at 3 and 12 months CA, iii) accurately identify infants at risk of adverse outcomes at an earlier stage, introducing an additional window of opportunity for intervention, iv) contribute to understanding brain development between 30 and 40 weeks PMA, v) and develop robust quantitative biomarkers of brain maturation, which can then be used in the research of interventions in this population.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JMG, RNB, PBC, SER, KP, MML, JF, BEL, SEB, CMF, AC, RSW contributed to study conception, design and development. PBC, RNB, SER and JMG achieved study funding. JMG, PBC, MML, AHTK, CMF, SEB will conduct the data acquisition. SER, JF and KP will perform MRI analysis. PBC, MML and AHTK will perform EEG analysis. JMG drafted the manuscript. All authors read, critically revised and approved the final manuscript.

Acknowledgements:

Grants from the Cerebral Palsy Alliance Research Foundation (IRG1413), the Financial Markets Foundation for Children (2014-074), the Queensland Government (Smart State; Health Practitioner Stimulus Grant) and the Royal Brisbane and Women's Hospital support this project. The authors received funding from The University of Queensland (University of Queensland Research Scholarship [JMG], Centennial Scholarship [KP], International Postgraduate Research Scholarship [KP]), the Queensland Government (Smart State PhD Top-Up Scholarship [JMG]), and the National Health and Medical Research council (Career Development Fellowship CDF-2 103887 [RNB]). We would like to acknowledge the families of preterm infants who participate in this study, the medical and nursing staff of the neonatal unit, radiology and administrative staff in the

department of medical imaging, and research nurses Donna Hovey and Kellie McGrory who undertake recruitment and study management.

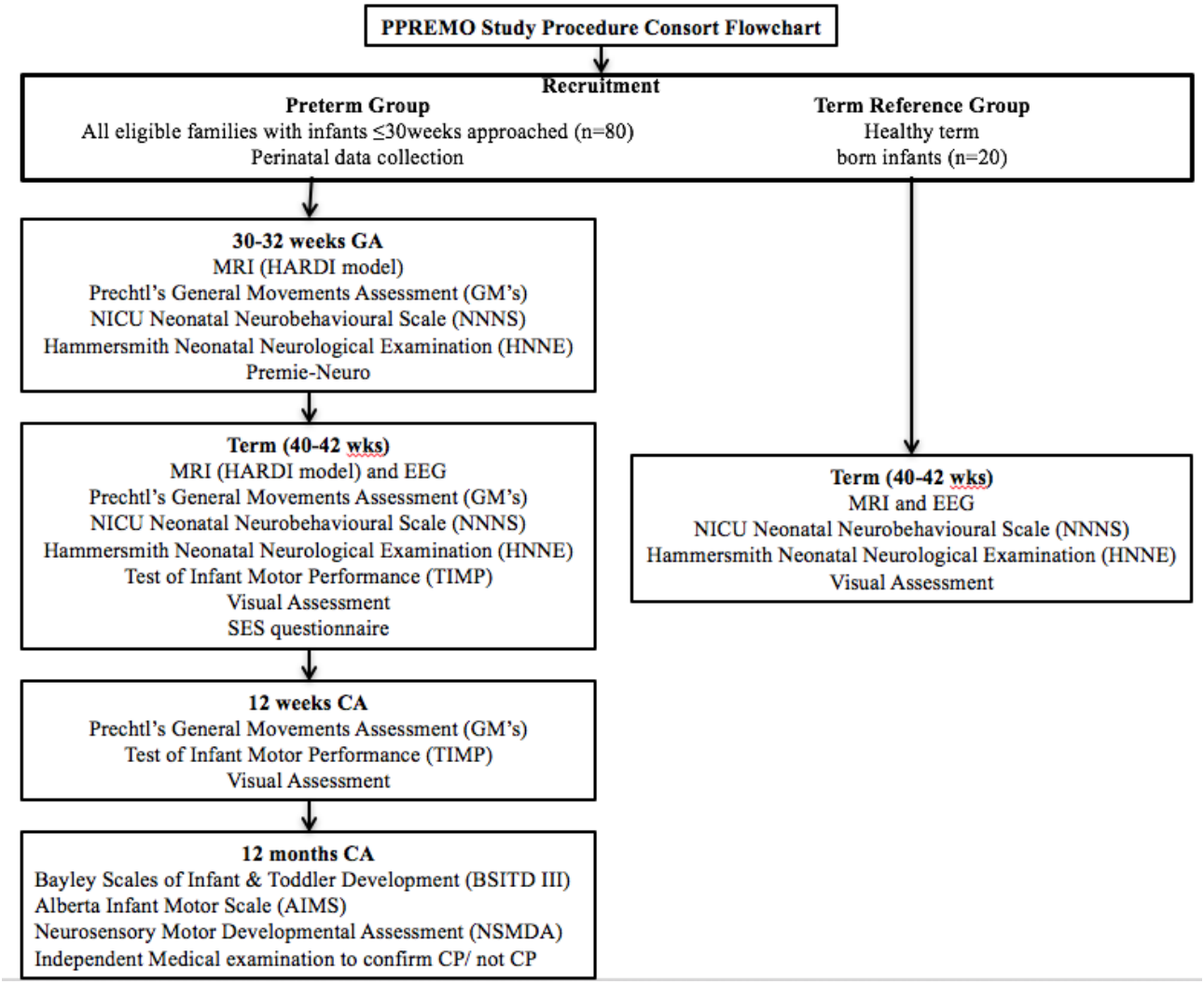
Clinical Trial Registration:

Australian New Zealand Clinical Trials Registry;

Trial Registration Number: ACTRN12613000280707;

Web address of trial: http://www.ANZCTR.org.au/ACTRN12613000280707.aspx

Figure 1: Consort Flowchart of PPREMO Study Procedure



Additional File 1: PPREMO Questionnaire - See Appendix 8

Additional file 2: PPREMO MR Protocol - See Appendix 9

References:

1. Doyle LW. Evaluation of neonatal intensive care for extremely low birth weight infants in Victoria over two decades: II. Efficiency. *Pediatrics* 2004; **113**: 510-4.

2. Holsti L, Grunau RVE, Whitfield MF. Developmental coordination disorder in extremely low birth weight children at nine years. *J Dev Behav Pediatr* 2002; **23**: 9.

3. Williams J, Lee KJ, Anderson PJ. Prevalence of motor-skill impairment in preterm children who do not develop cerebral palsy: a systematic review. *Dev Med Child Neurol* 2010; **52**: 232-7.

4. Mathur A, Inder T. Magnetic resonance imaging--insights into brain injury and outcomes in premature infants. *J Commun Disord* 2009; **42**: 248-55.

5. Mikkola K, Ritari N, Tommiska V, et al. Neurodevelopmental outcome at 5 years of age of a national cohort of extremely low birth weight infants who were born in 1996-1997. *Pediatrics* 2005; **116**: 1391-400.

6. Spittle AJ, Boyd RN, Inder TE, Doyle LW. Predicting motor development in very preterm infants at 12 months' corrected age: the role of qualitative magnetic resonance imaging and general movements assessments. *Pediatrics* 2009; **123**: 512-7.

7. Skiold B, Eriksson C, Eliasson AC, Aden U, Vollmer B. General movements and magnetic resonance imaging in the prediction of neuromotor outcome in children born extremely preterm. *Early Hum Dev* 2013; **89**: 467-72.

8. Woodward LJ, Anderson PJ, Austin NC, Howard K, Inder TE. Neonatal MRI to predict neurodevelopmental outcomes in preterm infants. *N Engl J Med* 2006; **355**: 685-94.

9. Spittle AJ, Cheong J, Doyle LW, et al. Neonatal white matter abnormality predicts childhood motor impairment in very preterm children. *Dev Med Child Neurol* 2011; **53**: 1000-6.

10. Bosanquet M, Copeland L, Ware R, Boyd R. A systematic review of tests to predict cerebral palsy in young children. *Dev Med Child Neurol* 2013; **55**: 418-26.

11. Nanba Y, Matsui K, Aida N, et al. Magnetic resonance imaging regional T1 abnormalities at term accurately predict motor outcome in preterm infants. *Pediatrics* 2007; **120**: e10-9.

12. van der Aa NE, Verhage CH, Groenendaal F, et al. Neonatal neuroimaging predicts recruitment of contralesional corticospinal tracts following perinatal brain injury. *Dev Med Child Neurol* 2013.

13. Krageloh-Mann I, Horber V. The role of magnetic resonance imaging in elucidating the pathogenesis of cerebral palsy: a systematic review. *Dev Med Child Neurol* 2007; **49**: 144-51.

14. Mirmiran M, Barnes PD, Keller K, et al. Neonatal brain magnetic resonance imaging before discharge is better than serial cranial ultrasound in predicting cerebral palsy in very low birth weight preterm infants. *Pediatrics* 2004; **114**: 992-8.

15. Dubois J, Benders M, Borradori-Tolsa C, et al. Primary cortical folding in the human newborn: an early marker of later functional development. *Brain* 2008; **131**: 2028-41.

16. Miller SP, Ferriero DM, Leonard C, et al. Early brain injury in premature newborns detected with magnetic resonance imaging is associated with adverse early neurodevelopmental outcome. *J Pediatr* 2005; **147**: 609-16.

17. Volpe JJ. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *Lancet Neurol* 2009; **8**: 110-24.

18. Arzoumanian Y, Mirmiran M, Barnes PD, et al. Diffusion tensor brain imaging findings at term-equivalent age may predict neurologic abnormalities in low birth weight preterm infants. *AJNR Am J Neuroradiol* 2003; **24**: 1646-53.

19. Rose J, Mirmiran M, Butler EE, et al. Neonatal microstructural development of the internal capsule on diffusion tensor imaging correlates with severity of gait and motor deficits. *Dev Med Child Neurol* 2007; **49**: 745-50.

20. Basser PJ, Mattiello J, LeBihan D. MR diffusion tensor spectroscopy and imaging. *Biophys J* 1994; **66**: 259-67.

21. Pierpaoli C, Jezzard P, Basser PJ, Barnett A, Di Chiro G. Diffusion tensor MR imaging of the human brain. *Radiology* 1996; **201**: 637-48.

22. Dubois J, Dehaene-Lambertz G, Perrin M, et al. Asynchrony of the early maturation of white matter bundles in healthy infants: quantitative landmarks revealed noninvasively by diffusion tensor imaging. *Hum Brain Mapp* 2008; **29**: 14-27.

23. Counsell SJ, Edwards AD, Chew AT, et al. Specific relations between neurodevelopmental abilities and white matter microstructure in children born preterm. *Brain* 2008; **131**: 3201-8.

24. Dudink J, Lequin M, van Pul C, et al. Fractional anisotropy in white matter tracts of very-low-birth-weight infants. *Pediatr Radiol* 2007; **37**: 1216-23.

25. De Bruine FT, Van Wezel-Meijler G, Leijser LM, et al. Tractography of white-matter tracts in very preterm infants: a 2-year follow-up study. *Dev Med Child Neurol* 2013; **55**: 427-33.

26. Krishnan ML, Dyet LE, Boardman JP, et al. Relationship between white matter apparent diffusion coefficients in preterm infants at term-equivalent age and developmental outcome at 2 years. *Pediatrics* 2007; **120**: e604-9.

27. Thompson DK, Inder TE, Faggian N, et al. Corpus callosum alterations in very preterm infants: perinatal correlates and 2 year neurodevelopmental outcomes. *Neuroimage* 2012; **59**: 3571-81.

28. Pannek K, Scheck SM, Colditz PB, Boyd RN, Rose SE. Magnetic resonance diffusion tractography of the preterm infant brain: a systematic review. *Dev Med Child Neurol* 2013.

29. Dubois J, Dehaene-Lambertz G, Kulikova S, Poupon C, Huppi PS, Hertz-Pannier L. The early development of brain white matter: a review of imaging studies in fetuses, newborns and infants. *Neuroscience* 2014; **276**: 48-71.

30. Pannek K, Guzzetta A, Colditz PB, Rose SE. Diffusion MRI of the neonate brain: acquisition, processing and analysis techniques. *Pediatr Radiol* 2012; **42**: 1169-82.

31. Raffelt D, Tournier JD, Rose S, et al. Apparent Fibre Density: a novel measure for the analysis of diffusion-weighted magnetic resonance images. *Neuroimage* 2012; **59**: 3976-94.

32. Arthurs OJ, Edwards A, Austin T, Graves MJ, Lomas DJ. The challenges of neonatal magnetic resonance imaging. *Pediatr Radiol* 2012; **42**: 1183-94.

33. Benavente-Fernandez I, Lubian-Lopez PS, Zuazo-Ojeda MA, Jimenez-Gomez G, Lechuga-Sancho AM. Safety of magnetic resonance imaging in preterm infants. *Acta Paediatr* 2010; **99**: 850-3.

34. Neubauer V, Griesmaier E, Baumgartner K, Mallouhi A, Keller M, Kiechl-Kohlendorfer U. Feasibility of cerebral MRI in non-sedated preterm-born infants at term-equivalent age: report of a single centre. *Acta Paediatr* 2011; **100**: 1544-7.

35. O'Regan K, Filan P, Pandit N, Maher M, Fanning N. Image quality associated with the use of an MR-compatible incubator in neonatal neuroimaging. *Br J Radiol* 2012; **85**: 363-7.

36. Lane A, Chuk LM, Colditz PB, Coulthard A. The MRI-compatible neonatal incubator in practice. *J Paediatr Child Health* 2013; **49**: E377-80.

37. Plaisier A, Raets MM, van der Starre C, et al. Safety of routine early MRI in preterm infants. *Pediatr Radiol* 2012; **42**: 1205-11.

38. Mrzljak L, Uylings HB, Kostovic I, Van Eden CG. Prenatal development of neurons in the human prefrontal cortex: I. A qualitative Golgi study. *J Comp Neurol* 1988; **271**: 355-86.

39. Bystron I, Blakemore C, Rakic P. Development of the human cerebral cortex: Boulder Committee revisited. *Nat Rev Neurosci* 2008; **9**: 110-22.

40. Chi JG, Dooling EC, Gilles FH. Gyral development of the human brain. *Ann Neurol* 1977; **1**: 86-93.

41. Bengoetxea H, Ortuzar N, Bulnes S, Rico-Barrio I, Lafuente JV, Argandona EG. Enriched and deprived sensory experience induces structural changes and rewires connectivity during the postnatal development of the brain. *Neural Plast* 2012; **2012**: 305693.

42. Kostovic I, Rakic P. Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. *J Comp Neurol* 1990; **297**: 441-70.

43. de Graaf-Peters VB, Hadders-Algra M. Ontogeny of the human central nervous system: what is happening when? *Early Hum Dev* 2006; **82**: 257-66.

44. Kostovic I, Jovanov-Milosevic N. The development of cerebral connections during the first 20-45 weeks' gestation. *Semin Fetal Neonatal Med* 2006; **11**: 415-22.

45. Kostovic I, Kostovic-Srzentic M, Benjak V, Jovanov-Milosevic N, Rados M. Developmental dynamics of radial vulnerability in the cerebral compartments in preterm infants and neonates. *Front Neurol* 2014; **5**: 139.

46. Boersma M, Smit DJ, de Bie HM, et al. Network analysis of resting state EEG in the developing young brain: structure comes with maturation. *Hum Brain Mapp* 2011; **32**: 413-25.

47. Gasser T, Jennen-Steinmetz C, Sroka L, Verleger R, Mocks J. Development of the EEG of school-age children and adolescents. II. Topography. *Electroencephalogr Clin Neurophysiol* 1988; **69**: 100-9.

48. Gasser T, Verleger R, Bacher P, Sroka L. Development of the EEG of school-age children and adolescents. I. Analysis of band power. *Electroencephalogr Clin Neurophysiol* 1988; **69**: 91-9.

49. Vanhatalo S, Kaila K. Development of neonatal EEG activity: from phenomenology to physiology. *Semin Fetal Neonatal Med* 2006; **11**: 471-8.

50. Zhu C, Guo X, Jin Z, et al. Influences of brain development and ageing on cortical interactive networks. *Clin Neurophysiol* 2011; **122**: 278-83.

51. El-Ayouty M, Abdel-Hady H, El-Mogy S, Zaghlol H, El-Beltagy M, Aly H. Relationship between electroencephalography and magnetic resonance imaging findings after hypoxic-ischemic encephalopathy at term. *Am J Perinatol* 2007; **24**: 467-73.

52. Wikstrom S, Pupp IH, Rosen I, et al. Early single-channel aEEG/EEG predicts outcome in very preterm infants. *Acta Paediatr* 2012; **101**: 719-26.

53. Klebermass K, Olischar M, Waldhoer T, Fuiko R, Pollak A, Weninger M. Amplitude-integrated EEG pattern predicts further outcome in preterm infants. *Pediatr Res* 2011; **70**: 102-8.

54. Riviello Jr JJ. Pediatric EEG Abnormalities. In: Blum AS, Rutkove SB, editors. The Clinical Neurophysiology Primer: Springer; 2007. 179-204.

55. Bell AH, McClure BG, McCullagh PJ, McClelland RJ. Variation in power spectral analysis of the EEG with gestational age. *J Clin Neurophysiol* 1991; **8**: 312-9.

56. Jenni OG, Borbely AA, Achermann P. Development of the nocturnal sleep electroencephalogram in human infants. *Am J Physiol Regul Integr Comp Physiol* 2004; **286**: R528-38.

57. Niemarkt HJ, Jennekens W, Pasman JW, et al. Maturational changes in automated EEG spectral power analysis in preterm infants. *Pediatr Res* 2011; **70**: 529-34.

58. Tolonen M, Palva JM, Andersson S, Vanhatalo S. Development of the spontaneous activity transients and ongoing cortical activity in human preterm babies. *Neuroscience* 2007; **145**: 997-1006.

59. Graziadio S, Basu A, Tomasevic L, Zappasodi F, Tecchio F, Eyre JA. Developmental tuning and decay in senescence of oscillations linking the corticospinal system. *J Neurosci* 2010; **30**: 3663-74.

60. Mathew P, Pannek K, Snow P, et al. Maturation of corpus callosum anterior midbody is associated with neonatal motor function in eight preterm-born infants. *Neural Plast* 2013; **2013**: 359532.

61. Pannek K, Hatzigeorgiou X, Colditz PB, Rose S. Assessment of structural connectivity in the preterm brain at term equivalent age using diffusion MRI and t2 relaxometry: a network-based analysis. *PLoS One* 2013; **8**: e68593.

62. Blankenship AG, Feller MB. Mechanisms underlying spontaneous patterned activity in developing neural circuits. *Nat Rev Neurosci* 2010; **11**: 18-29.

63. Omidvarnia A, Fransson P, Metsaranta M, Vanhatalo S. Functional bimodality in the brain networks of preterm and term human newborns. *Cereb Cortex* 2014; **24**: 2657-68.

64. Noble Y, Boyd R. Neonatal assessments for the preterm infant up to 4 months corrected age: a systematic review. *Dev Med Child Neurol* 2012; **54**: 129-39.

65. Spittle AJ, Doyle LW, Boyd RN. A systematic review of the clinimetric properties of neuromotor assessments for preterm infants during the first year of life. *Dev Med Child Neurol* 2008; **50**: 254-66.

66. Einspieler C. Prechtl's method on the qualitative assessment of general movements in preterm, term and young infants. Cambridge, UK: Mac Keith Press; 2004.

67. Bruggink JL, Cioni G, Einspieler C, Maathuis CG, Pascale R, Bos AF. Early motor repertoire is related to level of self-mobility in children with cerebral palsy at school age. *Dev Med Child Neurol* 2009; **51**: 878-85.

68. Bruggink JL, Van Braeckel KN, Bos AF. The early motor repertoire of children born preterm is associated with intelligence at school age. *Pediatrics* 2010; **125**: e1356-63.

69. Bruggink JL, Einspieler C, Butcher PR, Van Braeckel KN, Prechtl HF, Bos AF. The quality of the early motor repertoire in preterm infants predicts minor neurologic dysfunction at school age. *J Pediatr* 2008; **153**: 32-9.

70. Hadders-Algra M, Bouwstra H, Groen SE. Quality of general movements and psychiatric morbidity at 9 to 12 years. *Early Hum Dev* 2009; **85**: 1-6.

71. Lester BM, Miller RJ, Hawes K, et al. Infant neurobehavioral development. *Semin Perinatol* 2011; **35**: 8-19.

72. Brown NC, Inder TE, Bear MJ, Hunt RW, Anderson PJ, Doyle LW. Neurobehavior at term and white and gray matter abnormalities in very preterm infants. *J Pediatr* 2009; **155**: 32-8, 8 e1.

73. Pineda RG, Tjoeng TH, Vavasseur C, Kidokoro H, Neil JJ, Inder T. Patterns of altered neurobehavior in preterm infants within the neonatal intensive care unit. *J Pediatr* 2013; **162**: 470-6 e1.

74. Constantinou JC, Adamson-Macedo EN, Mirmiran M, Fleisher BE. Movement, imaging and neurobehavioral assessment as predictors of cerebral palsy in preterm infants. *J Perinatol* 2007; **27**: 225-9.

75. Stephens BE, Liu J, Lester B, et al. Neurobehavioral assessment predicts motor outcome in preterm infants. *J Pediatr* 2010; **156**: 366-71.

76. Romeo DM, Cioni M, Palermo F, Cilauro S, Romeo MG. Neurological assessment in infants discharged from a neonatal intensive care unit. *Eur J Paediatr Neurol* 2013; **17**: 192-8.

77. Palmer FB. Strategies for the early diagnosis of cerebral palsy. *J Pediatr* 2004; **145**: S8-S11.

78. Ferrari F, Cioni G, Einspieler C, et al. Cramped synchronized general movements in preterm infants as an early marker for cerebral palsy. *Arch Pediatr Adolesc Med* 2002; **156**: 460-7.

79. Lacey JL, Rudge S, Rieger I, Osborn DA. Assessment of neurological status in preterm infants in neonatal intensive care and prediction of cerebral palsy. *Aust J Physiother* 2004; **50**: 137-44.

80. Woodward LJ, Mogridge N, Wells SW, Inder TE. Can neurobehavioral examination predict the presence of cerebral injury in the very low birth weight infant? *J Dev Behav Pediatr* 2004; **25**: 326-34.

81. Cioni G, Ferrari F, Einspieler C, Paolicelli PB, Barbani MT, Prechtl HF. Comparison between observation of spontaneous movements and neurologic examination in preterm infants. *J Pediatr* 1997; **130**: 704-11.

82. Stoltz Sjostrom E, Ohlund I, Ahlsson F, et al. Nutrient intakes independently affect growth in extremely preterm infants: results from a population-based study. *Acta Paediatr* 2013; **102**: 1067-74.

83. Belfort MB, Rifas-Shiman SL, Sullivan T, et al. Infant growth before and after term: effects on neurodevelopment in preterm infants. *Pediatrics* 2011; **128**: e899-906.

84. Ehrenkranz RA, Dusick AM, Vohr BR, Wright LL, Wrage LA, Poole WK. Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. *Pediatrics* 2006; **117**: 1253-61.

85. Moltu SJ, Blakstad EW, Strommen K, et al. Enhanced feeding and diminished postnatal growth failure in very-low-birth-weight infants. *J Pediatr Gastroenterol Nutr* 2014; **58**: 344-51.

86. Morgan C, McGowan P, Herwitker S, Hart AE, Turner MA. Postnatal head growth in preterm infants: a randomized controlled parenteral nutrition study. *Pediatrics* 2014; **133**: e120-8.

87. Moyses HE, Johnson MJ, Leaf AA, Cornelius VR. Early parenteral nutrition and growth outcomes in preterm infants: a systematic review and meta-analysis. *Am J Clin Nutr* 2013; **97**: 816-26.

88. Romeo DM, Guzzetta A, Scoto M, et al. Early neurologic assessment in preterm-infants: integration of traditional neurologic examination and observation of general movements. *Eur J Paediatr Neurol* 2008; **12**: 183-9.

89. Setanen S, Lahti K, Lehtonen L, et al. Neurological examination combined with brain MRI or cranial US improves prediction of neurological outcome in preterm infants. *Early Hum Dev* 2014; **90**: 851-6.

90. Spittle AJ, Brown NC, Doyle LW, et al. Quality of general movements is related to white matter pathology in very preterm infants. *Pediatrics* 2008; **121**: e1184-9.

91. Lodygensky GA, Seghier ML, Warfield SK, et al. Intrauterine growth restriction affects the preterm infant's hippocampus. *Pediatr Res* 2008; **63**: 438-43.

92. Rose J, Vassar R, Cahill-Rowley K, et al. Neonatal physiological correlates of near-term brain development on MRI and DTI in very-low-birth-weight preterm infants. *NeuroImage Clinical* 2014; **5**: 169-77.

93. Inder TE, Warfield SK, Wang H, Huppi PS, Volpe JJ. Abnormal cerebral structure is present at term in premature infants. *Pediatrics* 2005; **115**: 286-94.

94. Orcesi S, Olivieri I, Longo S, et al. Neurodevelopmental outcome of preterm very low birth weight infants born from 2005 to 2007. *Eur J Paediatr Neurol* 2012; **16**: 716-23.

95. Chow SSW. Report of the Australian and New Zealand Neonatal Network 2012. Sydney: ANZNN; 2014.

96. Chawla S, Bapat R, Pappas A, Bara R, Zidan M, Natarajan G. Neurodevelopmental outcome of extremely premature infants exposed to incomplete, no or complete antenatal steroids. *J Matern Fetal Neonatal Med* 2013; **26**: 1542-7.

97. Roberts D, Dalziel S. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *The Cochrane database of systematic reviews* 2006: Cd004454.

98. Doyle LW, Crowther CA, Middleton P, Marret S, Rouse D. Magnesium sulphate for women at risk of preterm birth for neuroprotection of the fetus. *The Cochrane database of systematic reviews* 2009: Cd004661.

99. Abdel-Latif ME, Bajuk B, Ward M, Oei JL, Badawi N. Neurodevelopmental outcomes of extremely premature infants conceived after assisted conception: a population based cohort study. *Arch Dis Child Fetal Neonatal Ed* 2013; **98**: F205-11.

100. Gnanendran L, Bajuk B, Oei J, Lui K, Abdel-Latif ME. Neurodevelopmental outcomes of preterm singletons, twins and higher-order gestations: a population-based cohort study. *Arch Dis Child Fetal Neonatal Ed* 2014.

101. Doyle LW, Roberts G, Anderson PJ, Victorian Infant Collaborative Study G. Changing long-term outcomes for infants 500-999 g birth weight in Victoria, 1979-2005. *Arch Dis Child Fetal Neonatal Ed* 2011; **96**: F443-7.

102. Wood NS, Marlow N, Costeloe K, Gibson AT, Wilkinson AR. Neurologic and developmental disability after extremely preterm birth. EPICure Study Group. *N Engl J Med* 2000; **343**: 378-84.

103. Marlow N, Wolke D, Bracewell MA, Samara M. Neurologic and developmental disability at six years of age after extremely preterm birth. *N Engl J Med* 2005; **352**: 9-19.

104. Marlow N, Hennessy EM, Bracewell MA, Wolke D, Group EPS. Motor and executive function at 6 years of age after extremely preterm birth. *Pediatrics* 2007; **120**: 793-804.

105. Tolsa CB, Zimine S, Warfield SK, et al. Early alteration of structural and functional brain development in premature infants born with intrauterine growth restriction. *Pediatr Res* 2004; **56**: 132-8.

106. Regev RH, Reichman B. Prematurity and intrauterine growth retardation--double jeopardy? *Clin Perinatol* 2004; **31**: 453-73.

107. De Jesus LC, Pappas A, Shankaran S, et al. Outcomes of Small for Gestational Age Infants Born at <27 Weeks' Gestation. *J Pediatr* 2013.

108. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *J Pediatr* 1978; **92**: 529-34.

109. Salhab WA, Perlman JM, Silver L, Sue Broyles R. Necrotizing enterocolitis and neurodevelopmental outcome in extremely low birth weight infants <1000 g. *J Perinatol* 2004; **24**: 534-40.

110. Schlapbach LJ, Aebischer M, Adams M, et al. Impact of sepsis on neurodevelopmental outcome in a Swiss National Cohort of extremely premature infants. *Pediatrics* 2011; **128**: e348-57.

111. Wood NS, Costeloe K, Gibson AT, Hennessy EM, Marlow N, Wilkinson AR. The EPICure study: associations and antecedents of neurological and developmental disability at 30 months of age following extremely preterm birth. *Arch Dis Child Fetal Neonatal Ed* 2005; **90**: F134-40.

112. Spittle AJ, Treyvaud K, Doyle LW, et al. Early emergence of behavior and social-emotional problems in very preterm infants. *J Am Acad Child Adolesc Psychiatry* 2009; **48**: 909-18.

113. Hack M. Survival and neurodevelopmental outcomes of preterm infants. *J Pediatr Gastroenterol Nutr* 2007; **45** 141-2.

114. Short EJ, Klein NK, Lewis BA, et al. Cognitive and academic consequences of bronchopulmonary dysplasia and very low birth weight: 8-year-old outcomes. *Pediatrics* 2003; **112**: e359.

115. Hoekstra RE, Ferrara TB, Couser RJ, Payne NR, Connett JE. Survival and long-term neurodevelopmental outcome of extremely premature infants born at 23-26 weeks' gestational age at a tertiary center. *Pediatrics* 2004; **113**: e1-6.

116. Hack M, Fanaroff AA. Outcomes of children of extremely low birthweight and gestational age in the 1990s. *Semin Neonatol* 2000; **5**: 89-106.

117. Vohr BR, Wright LL, Dusick AM, et al. Neurodevelopmental and functional outcomes of extremely low birth weight infants in the National Institute of Child Health and Human Development Neonatal Research Network, 1993-1994. *Pediatrics* 2000; **105**: 1216-26.

118. Delobel-Ayoub M, Kaminski M, Marret S, et al. Behavioral outcome at 3 years of age in very preterm infants: the EPIPAGE study. *Pediatrics* 2006; **117**: 1996-2005.

119. Gidrewicz DA, Fenton TR. A systematic review and meta-analysis of the nutrient content of preterm and term breast milk. *BMC Pediatr* 2014; **14**: 216.

120. Treyvaud K, Ure A, Doyle LW, et al. Psychiatric outcomes at age seven for very preterm children: rates and predictors. *J Child Psychol Psychiatry* 2013; **54**: 772-9.

121. Hack M, Breslau N, Aram D, Weissman B, Klein N, Borawski-Clark E. The effect of very low birth weight and social risk on neurocognitive abilities at school age. *J Dev Behav Pediatr* 1992; **13**: 412-20.

122. Roberts G, Howard K, Spittle AJ, Brown NC, Anderson PJ, Doyle LW. Rates of early intervention services in very preterm children with developmental disabilities at age 2 years. *J Paediatr Child Health* 2008; **44**: 276-80.

123. Arpi E, Ferrari F. Preterm birth and behaviour problems in infants and preschool-age children: a review of the recent literature. *Dev Med Child Neurol* 2013; **55**: 788-96.

124. Solaski M, Majnemer A, Oskoui M. Contribution of socio-economic status on the prevalence of cerebral palsy: a systematic search and review. *Dev Med Child Neurol* 2014; **56**: 1043-51.

125. Brown N, Spittle A. Neurobehavioral evaluation in the preterm and term infant. *Curr Pediatr Rev* 2014; **10**: 65-72.

126. Van Hus JW, Jeukens-Visser M, Koldewijn K, et al. Comparing two motor assessment tools to evaluate neurobehavioral intervention effects in infants with very low birth weight at 1 year. *Phys Ther* 2013; **93**: 1475-83.

127. Kidokoro H, Neil JJ, Inder TE. New MR imaging assessment tool to define brain abnormalities in very preterm infants at term. *AJNR Am J Neuroradiol* 2013; **34**: 2208-14.

128. Epstein HT. Stages of increased cerebral blood flow accompany stages of rapid brain growth. *Brain Dev* 1999; **21**: 535-9.

129. Shi F, Yap PT, Wu G, et al. Infant brain atlases from neonates to 1- and 2-year-olds. *PLoS One* 2011; **6**: e18746.

130. Gousias IS, Edwards AD, Rutherford MA, et al. Magnetic resonance imaging of the newborn brain: manual segmentation of labelled atlases in term-born and preterm infants. *Neuroimage* 2012; **62**: 1499-509.

131. Gousias IS, Hammers A, Counsell SJ, et al. Magnetic resonance imaging of the newborn brain: automatic segmentation of brain images into 50 anatomical regions. *PLoS One* 2013; **8**: e59990.

132. Tournier JD, Calamante F, Connelly A. Robust determination of the fibre orientation distribution in diffusion MRI: non-negativity constrained super-resolved spherical deconvolution. *Neuroimage* 2007; **35**: 1459-72.

133. 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.

134. Sustersic B, Sustar K, Paro-Panjan D. General movements of preterm infants in relation to their motor competence between 5 and 6 years. *Eur J Paediatr Neurol* 2012; **16**: 724-9.

135. Butcher PR, van Braeckel K, Bouma A, Einspieler C, Stremmelaar EF, Bos AF. The quality of preterm infants' spontaneous movements: an early indicator of intelligence and behaviour at school age. *J Child Psychol Psychiatry* 2009; **50**: 920-30.

136. Kodric J, Sustersic B, Paro-Panjan D. Assessment of general movements and 2.5 year developmental outcomes: pilot results in a diverse preterm group. *Eur J Paediatr Neurol* 2010; **14**: 131-7.

137. Hadders-Algra M, Mavinkurve-Groothuis AM, Groen SE, Stremmelaar EF, Martijn A, Butcher PR. Quality of general movements and the development of minor neurological dysfunction at toddler and school age. *Clin Rehabil* 2004; **18**: 287-99.

138. Hadders-Algra M, Groothuis AM. Quality of general movements in infancy is related to neurological dysfunction, ADHD, and aggressive behaviour. *Dev Med Child Neurol* 1999; **41**: 381-91.

139. Lester BM, Tronick EZ, LaGasse L, et al. The maternal lifestyle study: effects of substance exposure during pregnancy on neurodevelopmental outcome in 1-month-old infants. *Pediatrics* 2002; **110**: 1182-92.

140. Lester BM, Tronick EZ, Brazelton TB. The Neonatal Intensive Care Unit Network Neurobehavioral Scale procedures. *Pediatrics* 2004; **113**: 641-67.

141. Tronick EZ, Olson K, Rosenberg R, Bohne L, Lu J, Lester BM. Normative neurobehavioral performance of healthy infants on the Neonatal Intensive Care Unit Network Neurobehavioral Scale. *Pediatrics* 2004; **113**: 676-8.

142. Fink NS, Tronick E, Olson K, Lester B. Healthy newborns' neurobehavior: norms and relations to medical and demographic factors. *J Pediatr* 2012; **161**: 1073-9.

143. Lester BM, Tronick EZ, LaGasse L, et al. Summary statistics of neonatal intensive care unit network neurobehavioral scale scores from the maternal lifestyle study: a quasinormative sample. *Pediatrics* 2004; **113**: 668-75.

144. Brown NC, Doyle LW, Bear MJ, Inder TE. Alterations in neurobehavior at term reflect differing perinatal exposures in very preterm infants. *Pediatrics* 2006; **118**: 2461-71.

145. El-Dib M, Massaro AN, Glass P, Aly H. Neurobehavioral assessment as a predictor of neurodevelopmental outcome in preterm infants. *J Perinatol* 2012; **32**: 299-303.

146. Liu J, Bann C, Lester B, et al. Neonatal neurobehavior predicts medical and behavioral outcome. *Pediatrics* 2010; **125**: e90-8.

147. Lester BM, Tronick EZ. History and description of the Neonatal Intensive Care Unit Network Neurobehavioral Scale. *Pediatrics* 2004; **113**: 634-40.

148. Dubowitz LM, Dubowitz V, Palmer P, Verghote M. A new approach to the neurological assessment of the preterm and full-term newborn infant. *Brain Dev* 1980; **2**: 3-14.

149. Dubowitz L, Mercuri E, Dubowitz V. An optimality score for the neurologic examination of the term newborn. *J Pediatr* 1998; **133**: 406-16.

150. Dubowitz LM, Dubowitz V, Mercuri E. Neurological Assessment of the Preterm and Fullterm Newborn Infant: Mac Keith Press; 1999.

151. Mercuri E, Guzzetta A, Laroche S, et al. Neurologic examination of preterm infants at term age: comparison with term infants. *J Pediatr* 2003; **142**: 647-55.

152. Ricci D, Romeo DM, Haataja L, et al. Neurological examination of preterm infants at term equivalent age. *Early Hum Dev* 2008; **84**: 751-61.

153. McGready R, Simpson J, Panyavudhikrai S, et al. Neonatal neurological testing in resource-poor settings. *Ann Trop Paediatr* 2000; **20**: 323-36.

154. Daily DK, Ellison PH. The premie-neuro: a clinical neurologic examination of premature infants. *Neonatal Netw* 2005; **24**: 15-22.

155. Gagnon K, Cannon S, Weatherstone KB. The premie-neuro: opportunities and challenges for standardized neurologic assessment of the preterm infant. *Adv Neonatal Care* 2012; **12**: 310-7.

156. Ricci D, Romeo DM, Serrao F, et al. Early assessment of visual function in preterm infants: how early is early? *Early Hum Dev* 2010; **86**: 29-33.

157. Ricci D, Cesarini L, Romeo DM, et al. Visual function at 35 and 40 weeks' postmenstrual age in low-risk preterm infants. *Pediatrics* 2008; **122**: e1193-8.

158. Ricci D, Romeo DM, Gallini F, et al. Early visual assessment in preterm infants with and without brain lesions: correlation with visual and neurodevelopmental outcome at 12 months. *Early Hum Dev* 2011; **87**: 177-82.

159. Mercuri E, Haataja L, Guzzetta A, et al. Visual function in term infants with hypoxic-ischaemic insults: correlation with neurodevelopment at 2 years of age. *Arch Dis Child Fetal Neonatal Ed* 1999; **80**: F99-104.

160. Campbell SK, Hedeker D. Validity of the Test of Infant Motor Performance for discriminating among infants with varying risk for poor motor outcome. *J Pediatr* 2001; **139**: 546-51.

161. Campbell SK, Kolobe TH, Osten ET, Lenke M, Girolami GL. Construct validity of the test of infant motor performance. *Phys Ther* 1995; **75**: 585-96.

162. Campbell SK, Kolobe TH, Wright BD, Linacre JM. Validity of the Test of Infant Motor Performance for prediction of 6-, 9- and 12-month scores on the Alberta Infant Motor Scale. *Dev Med Child Neurol* 2002; **44**: 263-72.

163. Kolobe TH, Bulanda M, Susman L. Predicting motor outcome at preschool age for infants tested at 7, 30, 60, and 90 days after term age using the Test of Infant Motor Performance. *Phys Ther* 2004; **84**: 1144-56.

164. Badawi N, Watson L, Petterson B, et al. What constitutes cerebral palsy? *Dev Med Child Neurol* 1998; **40**: 520-7.

165. Gainsborough M, Surman G, Maestri G, Colver A, Cans C. Validity and reliability of the guidelines of the surveillance of cerebral palsy in Europe for the classification of cerebral palsy. *Dev Med Child Neurol* 2008; **50**: 828-31.

166. Palisano RJ, Hanna SE, Rosenbaum PL, et al. Validation of a model of gross motor function for children with cerebral palsy. *Phys Ther* 2000; **80**: 974-85.

167. Bayley N. Bayley Scales of Infant and Toddler Development–Third Edition. San Antonio, TX: Harcourt Assessment; 2006.

168. Luttikhuizen Dos Santos ES, de Kieviet JF, Konigs M, van Elburg RM, Oosterlaan J. Predictive value of the Bayley Scales of Infant Development on development of very preterm/very low birth weight children: A meta-analysis. *Early Hum Dev* 2013; **89**: 487-96.

169. Burns YR, Ensbey RM, Norrie MA. The Neuro-Sensory Motor Developmental Assessment: Part 1. Development and administration of the test. *Aust J Physiother* 1989; **35**: 141-9.

170. Burns YR, Ensbey RM, Norrie MA. The Neuro Sensory Motor Developmental Assessment Part II: Predictive and concurrent validity. *Aust J Physiother* 1989; **35**: 151-57.

171. Burns Y, O'Callaghan M, McDonell B, Rogers Y. Movement and motor development in ELBW infants at 1 year is related to cognitive and motor abilities at 4 years. *Early Hum Dev* 2004; **80**: 19-29.

172. Danks M, Maideen MF, Burns YR, et al. The long-term predictive validity of early motor development in "apparently normal" ELBW survivors. *Early Hum Dev* 2012; **88**: 637-41.

173. Piper MC, Darrah J. Motor assessment of the developing infant: Saunders Alberta; 1994.

174. Piper MC, Pinnell LE, Darrah J, Maguire T, Byrne PJ. Construction and validation of the Alberta Infant Motor Scale (AIMS). *Can J Public Health* 1992; **83 Suppl 2**: S46-50.

175. van Haastert IC, de Vries LS, Helders PJ, Jongmans MJ. Early gross motor development of preterm infants according to the Alberta Infant Motor Scale. *J Pediatr* 2006; **149**: 617-22.

176. Blanchard Y, Neilan E, Busanich J, Garavuso L, Klimas D. Interrater reliability of early intervention providers scoring the alberta infant motor scale. *Pediatr Phys Ther* 2004; **16**: 13-8.

177. Jeng SF, Yau KI, Chen LC, Hsiao SF. Alberta infant motor scale: reliability and validity when used on preterm infants in Taiwan. *Phys Ther* 2000; **80**: 168-78.

178. Darrah J, Piper M, Watt MJ. Assessment of gross motor skills of at-risk infants: predictive validity of the Alberta Infant Motor Scale. *Dev Med Child Neurol* 1998; **40**: 485-91.

.

3.3 Summary of Chapter 3

This chapter presented the rationale, aims and methodology of the prospective cohort study within which this thesis is embedded. The literature review for the protocol paper was completed in 2014, and a substantial amount of new data has been published since then. Brain imaging of preterm infants in the neonatal period and up to TEA has gathered momentum over the last few years. This is evidenced by the number of peer-reviewed publications of MRI in preterm infants increasing exponentially from 27 in the year 2005 to 62 in 2010, 111 in 2015 and 135 in 2016 (data from pubmed). Chapter 2 of this thesis has summarised and presented the literature pertinent to this thesis up to March 2017, ensuring that all relevant published literature has been incorporated.

It must also be noted that academic discussions in recent years have drawn attention to, and cautioned against the use of the terms 'predict/s' in the context of MRI and the relationship with neurodevelopmental outcomes[24]. In addition, systematic reviews published since this protocol paper have shown sensitivity of MRI at TEA to predict CP ranging from 67-100%[25]. False positives are also noted at advanced NICU centres publishing optimally studied cohorts. For these reasons, while this thesis set out to evaluate the ability of MRI to predict CP, we have been careful not to overstate, and instead refer to MRI as 'determining/detecting' cases of adverse motor outcomes and CP, or 'demonstrating predictive value for' adverse motor outcomes and/or CP.

This thesis fits within the broader PPREMO study. Aims of this thesis as outlined in Chapter 1 are encompassed by overarching primary aims 1-3 from the protocol paper, but are more detailed and tailored to this thesis. The perinatal data related to primary aim 4 has been collected, and is used in this thesis for the purposes of describing the cohort and for use in multivariable regression models where required. Secondary aims focussing on EEG and nutritional intake are part of the broader PPREMO study.

3.3.1 Implementation of the PPREMO study

Recruitment commenced in February 2013 and was completed in April 2016. Final 12-month outcome data was collected in May 2017. The published study protocol asserted an aim to recruit a total sample size of 80 infants with full data sets. Various factors impacted the number of infants with full datasets, such as attrition or motion artefacts making diffusion MR images unusable for analysis. For these reasons, a total of 146 preterm infants were recruited. Of these, 119 infants underwent Early MRI and concurrent clinical assessments, 109 returned at term and 105 of these had a term MRI. Three month follow up was conducted for 107 infants and 104 had 12-month

outcome assessments completed. The PPREMO study also recruited a reference sample of 19 term born infants with MRI and clinical data, and had data sharing agreements with another 2 studies which provided a further 19 datasets. The total term reference sample had data from 38 infants.

The first step to understand the Early MRI data was to examine the structural MRI data. A validated scoring system for structural images was required. On examination of the literature it was found that the most comprehensive scoring system for use in very preterm infants at TEA was that by Kidokoro *et al*[26]. The Kidokoro scoring system combines evaluation of brain injury, with regional measures designed to capture the effect of secondary brain growth. It also evaluates deep GM and the cerebellum, and has been validated for use from 36-42 weeks PMA. Scoring systems for use earlier than TEA in preterm infants were available[10, 11], however they evaluated WM and cortical GM only. The decision was made to validate the Kidokoro scoring method for Early MRI (29-35) weeks.

The progression of the study and order of data analyses and preparation of publications which are included in this thesis are illustrated in Figure 1. The order in which these analyses were undertaken impacted the sample sizes available for each paper. Sample size calculations for the PPREMO study indicated that 80 infants with Early MRI and 12 month outcomes would provide adequate power for statistical analysis. As all structural MR images were able to be scored with the Kidokoro method, once the threshold of 80 datasets was reached, the validation paper was initiated. Subsequently, once the full cohort had been recruited and Early and Term MRI data collected, the relationships between Early structural MRI and concurrent clinical measures were evaluated. Once the final participant 12-month data had been collected, evaluation of diffusion MRI data was undertaken.

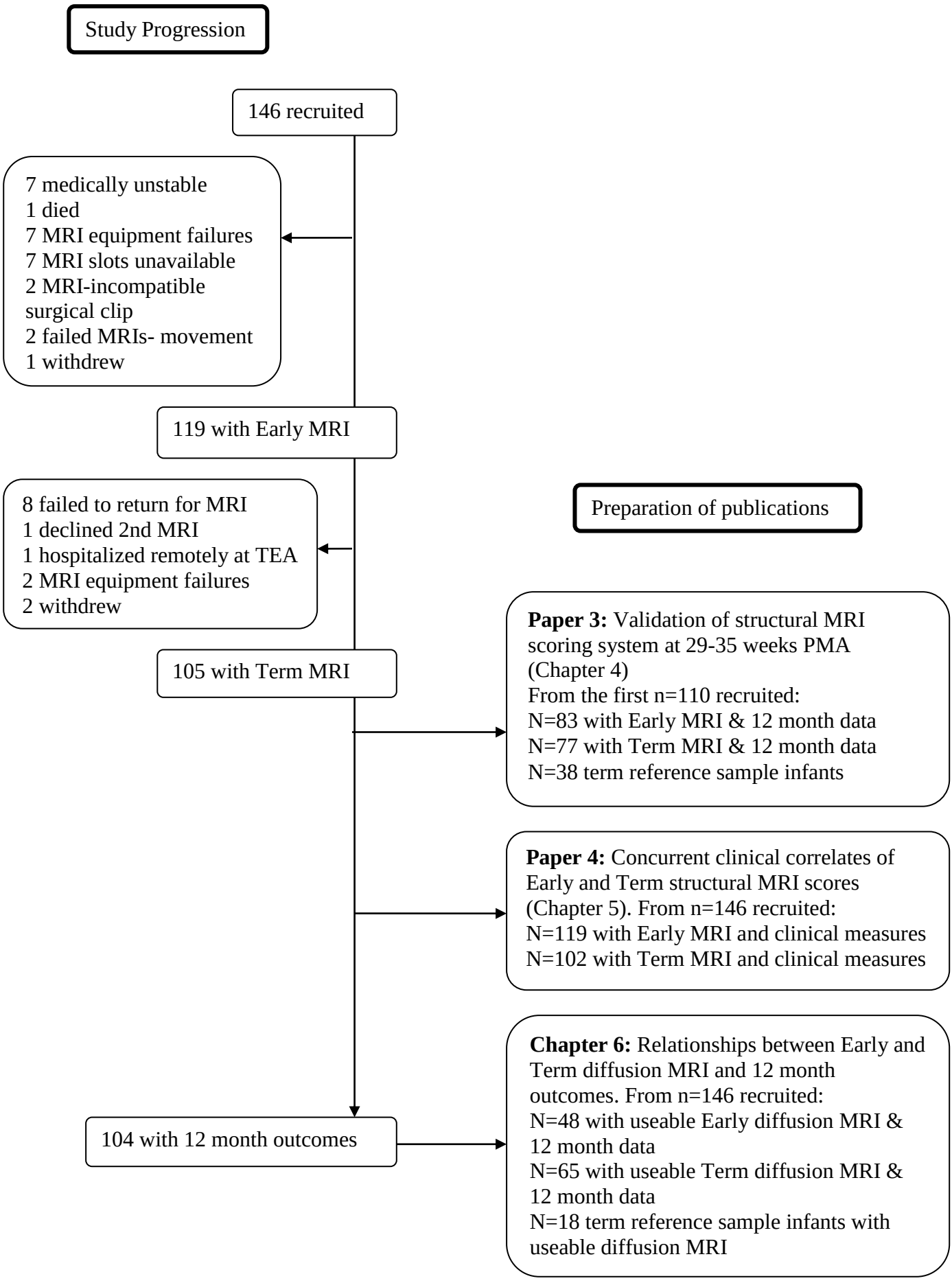


Figure 1: Study progression and preparation of publications

Chapter 4: Validation of an MRI brain injury and growth scoring system in very preterm infants scanned at 29-35 weeks postmenstrual age

4.1 Introduction to Chapter 4

The need for validated structural MRI scoring systems for use in the early period was believed to be an important first step in understanding the Early MRI data. The benefit to clinicians is that such tools are clinically accessible, relatively quick and easy to administer, and with validation with later outcomes, might provide diagnostic and prognostic information. From a research perspective, most studies involving advanced diffusion imaging at TEA utilise qualitative MRI scoring systems to describe their cohort, define inclusion and exclusion criteria or to correlate their advanced metrics with known structural abnormalities found on structural images. Diffusion studies of Early MRI require validated tools for analysis of structural images for the same purposes. An additional benefit is that all MRIs in the present cohort could be classified using a structural scoring system whereas advanced imaging excludes up to 40% of participant MRIs due to movement artefact or limitations with tissue segmentation.

4.2 Paper 3:

George JM, Fiori S, Fripp J, Pannek K, Bursle J, Moldrich RX, Guzzetta A, Coulthard A, Ware RS, Rose SE, Colditz PB, Boyd RN. Validation of an MRI Brain Injury and Growth Scoring System in Very Preterm Infants Scanned at 29- to 35-Week Postmenstrual Age. *AJNR Am J Neuroradiol* 2017: May 18. doi: 0.3174/ajnr.A5191 [Epub ahead of print].

Validation of an MRI Brain Injury and Growth Scoring System in Very Preterm Infants Scanned at 29- to 35-Week Postmenstrual Age

George JM, Fiori S, Fripp J, Pannek K, Bursle J, Moldrich RX, Guzzetta A, Coulthard A, Ware RS, Rose SE, Colditz PB, Boyd RN

Abstract

Background and Purpose The diagnostic and prognostic potential of brain MRI prior to term-equivalent age (TEA) is limited until valid MRI scoring systems are available. This study aimed to validate an MRI scoring system of brain injury and impaired growth for use at 29 to 35 weeks postmenstrual age in infants born <31weeks gestational age.

Materials and Methods Eighty-three infants in a prospective cohort study underwent Early 3T MRI between 29 and 35weeks postmenstrual age (mean 32^{+2} $\pm1^{+3}$weeks; 49 males, born at median gestation of 28^{+4}weeks; range, 23^{+6}-30^{+6}weeks; mean birthweight, 1068 ±312 g). Seventy-seven infants had a second MRI at term-equivalent age (mean, 40^{+6} $\pm1^{+3}$weeks). Structural images were scored using a modified scoring system which generated WM, cortical gray matter, deep gray matter, cerebellar and global scores. Outcome at 12 months corrected age (mean, 12 months 4 days $\pm1^{+2}$weeks) consisted of the Bayley Scales of Infant and Toddler Development, 3rd ed. (Bayley III), and the Neuro-Sensory Motor Developmental Assessment.

Results Early MRI global, WM, and deep gray matter scores were negatively associated with Bayley III motor (regression coefficient for global score ß=-1.31; 95%CI=-2.39, -0.23; p=0.02), cognitive (ß=-1.52; 95%CI=-2.39, -0.65; p<0.01) and the Neuro-Sensory Motor Developmental Assessment outcomes (ß=-1.73; 95%CI=-3.19, -0.28; p=0.02). Early MRI cerebellar scores were negatively associated with the Neuro-Sensory Motor Developmental Assessment (ß = -5.99; 95% CI, -11.82, -0.16; p = 0.04. Results were reconfirmed at term-equivalent age MRI.

Conclusion This clinically accessible MRI scoring system is valid for use at 29 to 35weeks postmenstrual age in infants born very preterm. It enables identification of infants at risk of adverse outcomes prior to the current standard of term-equivalent age.

Abbreviations:

c-, corrected; CGM, cortical gray matter; DGM, deep gray matter

Preterm infants are at risk of brain injury and impaired brain growth and consequently poorer outcomes in infancy and childhood[1-6]. Scoring of structural MRI to classify brain injury and growth has been validated for use at term-equivalent age (TEA) in infants born preterm[1, 7]. Initial systems were qualitative, focusing on classification of the severity of WM and cortical gray matter (CGM) injuries[7-9]. The degree of WM abnormality demonstrated significant associations with concurrent motor, neurological, and neurobehavioural performance[10-13], and increasing WM abnormality was associated with poorer motor and cognitive outcomes[1, 2, 5, 7, 14-16].

Scoring systems of MRI at TEA were further developed to include quantitative biometrics to measure the impact of secondary brain maturation and growth following preterm brain injury[17]. These brain metrics correlated with brain volumes and differentiated preterm and term-born infants at TEA MRI[17]. At TEA, transcerebellar diameter was associated with fidgety general movements at 3 months corrected age (CA)[18], poorer cognitive outcomes at 12 months CA[19], and poorer motor and cognitive outcomes at 2 years CA[20]. Reduced deep gray matter area at TEA was associated with poorer motor and cognitive outcomes[19] and an increased interhemispheric distance independently predicted poorer cognitive development at 2 years CA[3]. Reduced biparietal width at TEA predicted both motor and cognitive outcomes at 2 years CA in infants born very preterm[3, 21].

Term-equivalent age MRI scoring systems have been further developed to include evaluation of deep gray matter (DGM) structures and the cerebellum[22]. At TEA, global brain abnormality scores were significantly associated with motor outcomes at 2 years CA[23] and cognitive outcomes at 7 years[24, 25]. Deep gray matter scores were significantly associated with poorer attention and processing speeds, memory and learning[24, 25].

With safe earlier MRI now possible using MR compatible incubators, valid scoring systems for use earlier than TEA are required. The aim of this study was to validate an MRI scoring system previously developed for very preterm infants at TEA in a cohort of infants born <31 weeks gestational age with MRI between 29 and 35 weeks PMA[22]. The study aimed to establish predictive validity for motor and cognitive outcomes at 12 months CA. Secondary aims were to examine inter- and intrarater reproducibility and to examine relationships between global brain abnormality categories and known perinatal risk factors. It was hypothesized that the scoring system would be valid and reliable for use at this earlier time point but with more infants classified with brain abnormalities, due to immaturity rather than injury.

Methods

Study Design and Participants

This prospective cohort study of infants born <31 weeks' gestational age (GA) was conducted at the Royal Brisbane and Women's Hospital, Brisbane, Australia between February 2013 and April 2015. Preterm infants were eligible if they had no congenital abnormality, and their parents/carers were English speaking who lived within a 200km radius of the hospital[26]. A reference sample of healthy term-born babies was simultaneously recruited to generate reference values and cut points for the regional brain measurements that form part of the scoring system. Inclusion criteria for term born infants were a GA at birth of 38-41 weeks, birthweight above the 10th percentile, an uncomplicated pregnancy, delivery and postpartum period, and normal neurological examination findings[26]. Ethics approval was obtained from the Royal Brisbane and Women's Hospital Human Research Ethics Committee (HREC/12/QRBW/245) and The University of Queensland (2012001060), and the trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12613000280707).

MRI Acquisition

Brain MRI was performed during sleep without sedation between 30-32 weeks PMA or when the infant was medically stable (range 29-35 weeks PMA, 'Early MRI') and again at TEA (40-42 weeks PMA, 'Term MRI'). Infants were scanned using a 3T MRI, Siemens Tim Trio (Erlangen, Germany), utilizing an MR compatible incubator with its dedicated neonatal head coil (Nomag incubator; LMT Lammers Medical Technology, Lübeck, Germany). Coronal, axial, and sagittal T2-weighted HASTE (TR/TE 2000/90ms, flip angle 150°, field of view 200x160mm, matrix 320x256, section thickness 4mm), axial T1 TSE (TR/TE 1490/90ms, flip angle 150°, field of view 200x160mm, matrix 256x180, section thickness 2mm), and an axial multi-echo T2 TSE (TR/TE1/TE2/TE3 10580/27/122/189ms, flip angle 150°, field of view 144x180mm, matrix 204x256, section thickness 2mm) were acquired.

MRI scoring

A standardized MRI scoring system according to Kidokoro *et al* was used to score all MRIs[22]. An independent neurologist with training in radiology and experienced in neonatal MRI scoring (SF) performed the scoring. The scorer had no knowledge of any clinical characteristics of the infants except PMA at the time of scanning. Scoring was confirmed by a senior neuroradiologist (AC). Modifications to scoring cut points were made using the term reference data means and standard deviations[27, 28]. Scoring items and parameters are detailed in On-line Table 1, a scoring proforma is

included in On-line Table 2, and On-line Figs 1-18 provide examples of lesion types and regional measurements.

Cerebral WM abnormality was rated on 6 components, with a maximum total score of 15: cystic degeneration, focal signal abnormalities, delayed myelination, thinning of the corpus callosum, dilated lateral ventricles, and reduction of WM volume[22]. Myelination of the corpus callosum and posterior limb of the internal capsule was expected by 36 weeks PMA, so all infants were given a score of 2 for this item on Early MRI. The CGM was rated on 3 components with a maximum total score of 8: signal abnormality, delayed gyration, and dilated extracerebral CSF space. Cerebellar and DGM abnormality were rated on signal abnormality and volume reduction with maximum total scores of 6 for each[22]. A total of WM, CGM, DGM and cerebellar scores yielded a global brain abnormality score (0-35)[22]. Each of the WM, CGM, DGM, cerebellum and global scores could be further categorized into no, mild, moderate or severe brain abnormality categories[22]. The WM total scores were categorized as none (0-2), mild (3-4), moderate (5-6) or severe (≥7) WM abnormality. Cortical GM, DGM and cerebellar categories used the following total scores; none (0), mild (1), moderate (2) and severe (≥3). Total global scores were classified as normal (0-3), mild (4-7), moderate (8-11) or severe (≥12) brain abnormalities.

Six regional measurements form part of the scoring: thickness of the corpus callosum (genu, body and splenium), ventricular diameter, biparietal width, interhemispheric distance, DGM area and transcerebellar diameter. These measurements change with PMA at time of MRI as a result of head and brain growth. To address this change and to minimize the risk of confounding, the relationship of each of these measures with PMA at MRI was examined to derive a correction method for PMA at MRI. The PMA was determined based on the obstetric estimate measure of gestation at delivery[29]. In the preterm group, Early and Term MRI data were pooled for each of the regional measures, and cases with focal brain lesions were removed to ensure that any linear relationship found was the result of age and not confounded by brain injury. For each measure that demonstrated a linear relationship with PMA at MRI, the regression coefficient (slope) was utilized to generate an equation for correction, written as: corrected value=measured value + regression coefficient x (40 - PMA at MRI). The correction was then applied to the full cohort. On-line Figs 8-10 and 15 provide instructions for conducting regional measurements, correcting the raw values and scoring.

The regional measurements were also obtained for the term reference sample and examination of the relationship with PMA at MRI was performed separately to that of the preterm group. When linear relationships were found, measurements were corrected as per the equation above. Following

correction of the term reference sample regional scores, means and standard deviations were calculated, and these were used to create cut points for scoring each of the respective regional measurements.

Inter-rater reproducibility of MRI scoring was tested on a separate sample with 20 MRI scans from each time point scored by a second blinded rater, a pediatric radiologist (JB). Intra-rater reproducibility was tested with 20 MRI scans from each time point rescored 1 month apart (SF).

Neurodevelopmental Outcome at 12 months CA

All infants underwent neurodevelopmental assessment at 12 months CA by an experienced physiotherapist blinded to MRI findings and medical history. The Bayley Scales of Infant and Toddler Development, 3rd ed. (Bayley III), was performed, and composite scores for motor and cognitive performance were generated[30]. The Neuro-Sensory Motor Developmental Assessment (NSMDA) evaluates neurological and sensory motor function in addition to gross and fine motor performance, with total scores and functional classifications used[31, 32]. The NSMDA at 12 months CA has good predictive validity for motor and cognitive outcomes and cerebral palsy at 4 years CA for very preterm infants[33, 34] and 24-month motor and functional outcomes for infants with cerebral palsy[35].

Statistical Analysis

Sample size calculations were based on qualitative evaluation of MR images at TEA predicting 12 month outcomes[4], with 69 infants required to reject the null hypothesis with 90% power (at $p<0.05$). A sample of 80 infants was recruited to account for attrition and the earlier PMA at MRI (29-35 weeks PMA).

The association between each of the 6 regional measurements and PMA at MRI was analyzed by using mixed effects regression models for the preterm sample data, and separately for the term reference sample data with linear regression. When a linear relationship was found, data were centered around the mean and the relationship was examined to determine if it was quadratic. Correction equations were then applied to the raw regional measures. Term reference sample mean and standard deviation data were used to generate scoring cut points for each of the regional measures. Paired *t*-tests were used to determine statistically significant differences between Early and Term MRI item scores in the preterm group.

The association between a) Early MRI scores and 12 month outcomes, and b) Term MRI scores and 12 month outcomes, were evaluated with univariable and multivariable linear regression. Multivariable regression included potential confounders of sex, social risk and, for NSMDA only, CA at assessment.

To examine the predictive validity of both Early and Term MRI, sensitivity, specificity and accuracy (percentage of cases correctly classified) were calculated. Dichotomized MRI and outcome data were used to construct 2x2 tables. MRI category scores were dichotomized into normal/mild or moderate/severe categories for each of the subscales and global scores. Bayley motor and cognitive composite scores were dichotomized (by <-1 SD) and the NSMDA functional classification scores as normal/minimal vs mild/moderate/severe/profound.

Inter- and intra-rater reliability was evaluated by using intra-class correlation coefficients (ICCs) (type 3,1). Agreement was evaluated using the percentage level of accuracy, in which the definition for accuracy was exact score ±1 for the subscale scores and exact score ±2 for the global scores.

When investigating perinatal risk factors, differences across global brain abnormality score categories were determined using Mann-Whitney *U* tests (dichotomous perinatal risk factors) and Kruskal Wallis 1-way ANOVAs (continuous perinatal risk factors). Analysis was performed using the Stata statistical package, version 14 (StataCorp, College Station, TX, USA).

Results

Participants

Of 214 eligible preterm infants, 110 consented to the study, of whom 83 had Early MRI and 12 month outcomes available and were included in this analysis (16 no Early MRI: 5 medically unstable, 1 death, 4 cancellations due to MRI equipment failure, 3 with no MRI slots, 1 withdrawn, 2 with movement artefacts; 11 failed to return for 12 month follow up). Of these, 77/83 had a second MRI at Term. Thirty-eight term born infants were included in the reference sample. Demographic data and MRI scores are summarized in Tables 1-3; 12 month outcomes are summarized in Table 4. There were minimal differences between those participants with both Early and Term MRI, and those with only Early MRI, except that all 6 participants who did not undergo their Term MRI were classified with a higher social risk[36, 37]. Given the established relationship between higher social risk and poorer neurodevelopmental outcome and an increased risk of cerebral palsy, and to address this difference in our cohort between Early and Term MRI, all

multivariable analyses included social risk as a potential confounder[38, 39]. All term reference sample infants had a normal global brain abnormality category score.

Associations between regional brain measurements and PMA at MRI

All preterm regional measures except the body of the corpus callosum demonstrated linear relationships with PMA at MRI ($p<0.01$). In the term reference sample, linear relationships were found only for transcerebellar diameter and corpus callosum genu. Results of regression analyses and corrected regional measures for the Early, Term and term reference sample MRIs are presented in On-line Tables 3 and 4.

Findings in each scoring domain at Early and Term MRI

Results for scoring items are presented in On-line Table 1. Incidence of WM cystic lesions, CGM signal abnormality, and WM volume reduction as measured by corrected biparietal width remained stable between Early and Term MRI. A proportion of signal abnormalities in the WM and DGM resolved between Early and Term MRI. A propensity to score worse at Term compared with Early MRI was evidenced for each of the following: ventricular dilatation, interhemispheric distance, volume reduction of DGM and cerebellum, and thinning of the corpus callosum. More infants had delayed gyral maturation at Early MRI compared with Term MRI.

Predictive validity of Early MRI

Results of univariable and multivariable regression analyses between Early MRI scores and neurodevelopmental outcomes are presented in Fig1 (first row); sensitivity, specificity and accuracy in Table 5. Global, WM and DGM scores on Early MRI were associated with Bayley III motor outcome multivariably (global score: β=-1.31; 95%CI=-2.39; -0.23; p=0.02). Early MRI WM, DGM and global scores were associated with Bayley III cognitive outcome (global β=-1.52; 95%CI=-2.39; -0.65; p<0.01). Early MRI WM, DGM, cerebellar and global scores were associated with outcome on the NSMDA (global β=-1.73; 95%CI=-3.19; -0.28; p=0.02). The sensitivity of Early MRI global scores to predict motor, cognitive, and NSMDA outcomes ranged from 33-50% specificity ranged from 86-87%, with the percentage of accurately classified cases ranging from 77-83%.

Predictive validity of term MRI

Univariable and multivariable regression analyses between Term MRI scores and neurodevelopmental outcomes are presented in Figure 1 (second row). Sensitivity, specificity and accuracy are presented in Table 5. At Term MRI, WM, DGM, cerebellar, and global scores were

associated with Bayley III motor and cognitive and NSMDA scores. Global scores were associated with Bayley III motor outcome (β =-1.71; 95%CI=-2.63; -0.79; p<0.01), cognitive outcome (β =-1.32; 95%CI=-2.10; -0.53; p<0.01), and NSMDA (β =-2.36; 95%CI=-3.62; -1.10; p<0.01). The sensitivity of Term MRI global scores to predict motor, cognitive and NSMDA outcomes ranged from 14-33% specificity ranged from 90-92% with the percentage of accurately classified cases ranging from 77-87%.

Inter- and intrarater reproducibility

Reliability and agreement results are presented in On-line Table 5. At Early MRI, intrarater reliability ranged from 0.82-0.97 (ICC), and agreement, from 90-100%. Interrater reliability was low for CGM (ICC=0.08), but excellent for the other subscales (ICC=0.76-0.86) and the global total (ICC=0.89). Interrater agreement ranged from 70-95%. At Term MRI, intrarater reliability ICCs ranged from 0.74-0.96, with global score ICC of 0.97. Intrarater agreement ranged from 90-100%. Interrater reliability once again showed lower reliability for CGM (ICC=0.66); however the other subscale ICCs ranged from 0.86-0.93, and the global score ICC was 0.93. Interrater agreement ranged from 80-100%.

Perinatal risk factors

Perinatal risk factors were associated with increasing severity of MRI global brain abnormality category scores (On-line Table 6). Early MRI was associated with gestational age at birth, birth weight, patent ductus arteriosus, retinopathy of prematurity, postnatal corticosteroids, ventilation, and oxygen therapy. Term MRI was associated with gestational age at birth, birth weight, higher social risk, retinopathy of prematurity, ventilation, oxygen requirement at 36 weeks PMA and requirement for home oxygen.

Discussion

This clinically accessible scoring system of structural brain MRI for use at 29-35 weeks PMA for infants born at <31 weeks gestational age is valid. Early MRI WM, DGM and global brain abnormality scores were associated with Bayley III motor and cognitive scores and outcome on the NSMDA at 12 months CA. Early cerebellar scores were also associated with NSMDA outcome. These associations were reconfirmed at Term MRI. In addition, Term MRI cerebellar scores were associated with Bayley III motor and cognitive outcomes.

Early MRI was more strongly associated with cognitive than motor outcomes. The scoring system upon which this study was based has been used in 2 studies examining the relationships between

TEA MRI and cognitive outcomes at 7 years[24, 25]. Our results support previous findings at TEA and suggest that the brain changes associated with adverse cognitive outcomes are already present as early as 29-35 weeks PMA[7].

Of all MRI subscale scores, at Early and Term MRI, DGM demonstrated the strongest relationship with outcome. This finding supports inclusion of DGM evaluation in qualitative and semi-quantitative scoring systems in this population. Cerebellar scores on Early MRI were associated with NSMDA scores but not the Bayley III motor score. This finding is interesting because the Bayley III motor scale focuses on motor achievement, while the NSMDA evaluates quality of motor performance, including balance and postural reactions, functions known to be modulated by the cerebellum. The NSMDA also includes assessment of muscle tone, reflexes and sensory motor function, and at 12 months CA has been shown to predict motor and cognitive outcomes and cerebral palsy at 4 years in preterm infants[33, 34].

The specificity of the scoring system is reasonable, indicating that those infants whose global scoring category is moderate or severe have a high probability of poor motor and cognitive outcomes at 12 months CA. The sensitivity is relatively low, so not all infants who progress to poor motor and cognitive outcomes will be identified by this scoring system at Early or Term MRI; however, it also means that the risk of false positives is low. Parents indicate a desire for prognostication and early identification of outcomes[40], and a low false positive rate is preferable to prolonged distress caused by a false positive result where parents spend years waiting for an adverse outcome that does not occur[41, 42]. A combination of TEA MRI findings and 3 months CA general movements assessment demonstrates improved predictive validity over TEA MRI alone[43-45], so evaluation of the relationships between this Early MRI scoring system and concurrent clinical measures and the combination of Early MRI and clinical measures to predict later outcomes is warranted.

Our results indicate that Term MRI scores demonstrate stronger associations with 12 month outcomes than Early MRI scores. Term MRI associations described here are stronger than those found by another group using the original scoring system[23], suggesting that the modified scoring cut points, based on term born reference sample data, may be an improvement over the original scale[27]. It must be noted that their outcome was at 2 years CA rather than 12 months CA in the present study. Stronger associations of Term MRI with outcomes may be due to small focal lesions evident on Early MRI having resolved by Term MRI, or volume reduction becoming more apparent. Both of these require further exploration. Term MRI scores presented here show a lower incidence of

myelination delay compared with the cohort upon which the scale was originally based. In the present study, the T1 sequence was performed at the end of the MRI when infants were often beginning to wake up; therefore, it had a higher incidence of motion artefacts. For this reason, T2-weighted images were used to score myelination delay with their improved contrast, and this may have resulted in an overestimation of myelination compared to the earlier study[22].

Conclusion

This study presents a clinically accessible MRI scoring system of brain injury and growth for use from 29-35 weeks PMA in infants born at <31 weeks GA that has good reproducibility and significant associations with motor and cognitive outcomes at 12 months CA. The tool is suitable for use in research and for assisting clinical patient management.

Acknowledgements

We acknowledge the families of infants who participated in this study; the staff of the neonatal unit and department of medical imaging; study personnel Dr Melissa Lai, Donna Hovey, Kellie McGrory, Kylie Smart, Christine Finn, Kym Morris; and Dr Francisco Perales for statistical support.

Table 1: Characteristics of preterm sample and term reference sample included in this validation study

	Full preterm sample with Early MRI n=83	**Preterm sample with additional Term MRI n=77**	**Term Reference Sample n=38**
Birth and Maternal Data	**n(%), Median[IQR] or Mean(SD), range**		
Gestational age at birth (weeks)	28^{+4}[26^{+6}-29^{+3}], 23^{+6} – 30^{+6}	28^{+5}[26^{+6}-29^{+3}], 23^{+6} – 30^{+6}	39^{+6}[39-40^{+3}], 38^{+2} – 41^{+3}
Birth weight (g)	1068(312), 494 – 1886	1076(322), 494 – 1886	3509(317), 2932 – 4330
Birth head circumference (cm)	25.62(2.38), 20.5-30.5 n=80	25.64(2.43), 20.5-30.5 n=75	34.71(1.12), 32.5 – 37 n=31
Males	49(59%)	46(60%)	19(50%)
Multiple births	24(29%)	21(27%)	0(0%)
Premature rupture of membranes	19(23%)	18(23%)	4(12%) n=33
Caesarian section	60(72%)	56(73%)	9(27%) n=33
Chorioamnionitis	14(17%)	13(17%)	
Antenatal steroids	62(75%)	57(74%)	
Magnesium sulphate	43(65%) n=66	41(65%) n=63	
Higher social risk	40(48%)	34(44%)	5(16%) n=31

Key: Early MRI, 29-35 weeks PMA; Term MRI, 40-42 weeks PMA; PMA postmenstrual age

Table 2: Characteristics of preterm sample and term reference sample included in this validation study

Acquired medical factors	From birth to Early MRI n=83	From birth to Term MRI n=77	Term Reference Sample n=38
Patent ductus arteriosus	39(47%)	36(47%)	
IVH	17(20%)	16(21%)	
IVH grade 3 or 4	4(5%)	4(5%)	
Periventricular leukomalacia	2(2%)	2(3%)	
Hydrocephalus	2(2%)	2(3%)	
NEC diagnosed or suspected	3(4%)	2(3%)	
Confirmed sepsis	3(4%)	2(3%)	
Total parenteral nutrition (days)	11[8–14], 0–30	11[8-14], 0–30	
Postnatal corticosteroids	14(17%)	14(18%)	
Ventilation (days)	3[0–12], 0–48	2[0–12], 0–48	
CPAP (days)	15[7–25], 0–47	30[7–47], 0–81	
Oxygen therapy (hours)	12[1–125], 0–1515, n=69	29[2–370], 0–3912, n=67	
36week PMA O_2 requirement		23(30%)	
PMA at MRI (weeks)	$32^{+2}(1^{+3})$, 29^{+3} –35^{+2}	$40^{+6}(1^{+3})$, 38^{+3}– 46^{+4}	$41^{+3}(1)$, 39^{+2} – 44
Weight at MRI (g)	1500(352), 883 – 2715	Early MRI 1505(359), 883-2715 Term MRI 3127(627), 1900–5150	3428(378), 2500–4200 n=31

Key: Early MRI, 29-35 weeks PMA; Term MRI, 40-42 weeks PMA; PMA postmenstrual age; IVH Intraventricular hemorrhage; NEC necrotizing enterocolitis; CPAP continuous positive airway pressure.

Table 3: Characteristics of preterm samples and term reference sample included in this validation study

MRI Scores	Early MRI	Term MRI	Term Reference Sample
		median[IQR]	
White Matter	3[2–4]	1[1–3]	0[0-0]
Cortical gray matter	0[0-1]	0[0-1]	0[0-0]
Deep gray matter	0[0-1]	0[0-1]	0[0-0]
Cerebellum	0[0-0]	0[0-1]	0[0-0]
Global Score	4[3–7]	3[1–5]	0[0-0]

Key: Early MRI, 29-35 weeks PMA; Term MRI, 40-42 weeks PMA; PMA postmenstrual age

Table 4: Bayley III and NSMDA scores at 12 months corrected age (n=83)

	12 month outcomes mean(SD)
Age at assessment	12 months 4 days (1^{+2}weeks)
Bayley III Motor Composite	96.96(14.27)
Bayley III Cognitive Composite	104.64(12.07)
NSMDA total	179.53(18.81)
12 month outcomes dichotomized number (%)	
Bayley III Motor Composite <-1SD	15(18%)
Bayley III Cognitive Composite <-1SD	6(7%)
NSMDA functional classification ≥2	7(8%)

Key: NSMDA neurosensory motor developmental assessment

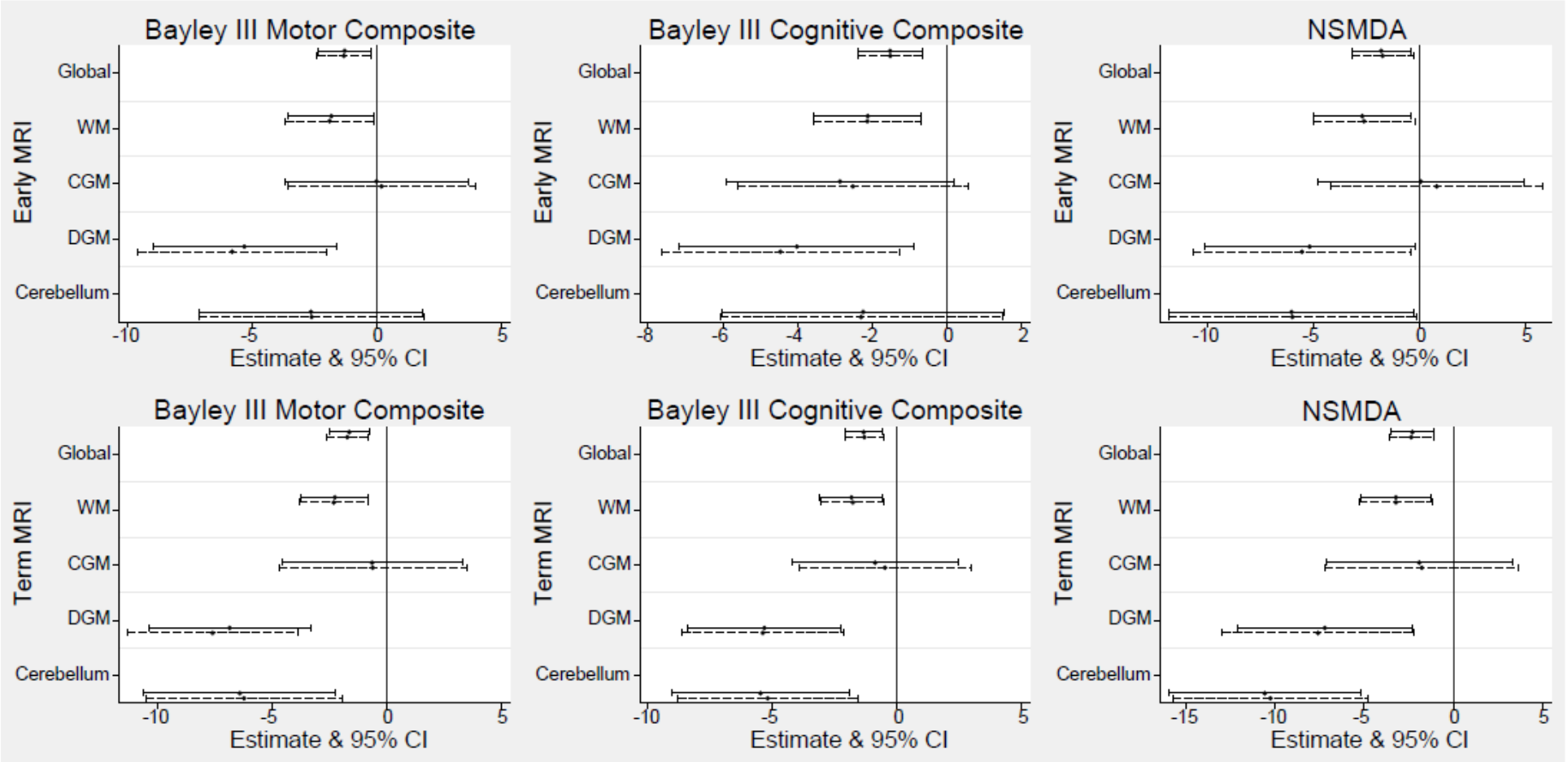


FIG 1. Associations between Early (first row) and Term (second row) MR imaging scores and neurodevelopmental outcome at 12-months corrected age for the preterm cohort. Solid lines represent univariable regression analyses, and dashed lines represent multivariable analyses for which sex, social risk and, for NSMDA only, corrected age at assessment were added.

Table 5: Sensitivity, specificity and accuracy of Early and Term MRI scores categorized as moderate/severe to predict an outcome of <-1SD on the Bayley III or NSMDA functional classification of mild-profound dysfunction

	12 month neurodevelopmental outcome								
	Bayley III Motor Composite score			Bayley III Cognitive Composite score			NSMDA		
Early MRI (n=83)	**Sensitivity**	**Specificity**	**Correctly classified**	**Sensitivity**	**Specificity**	**Correctly classified**	**Sensitivity**	**Specificity**	**Correctly classified**
WM	33(12-62)	78(66-87)	70	50(12-88)	78(67-87)	76	43(10-82)	78(67-86)	75
CGM	0(0-22)	81(70-89)	66	0(0-46)	83(73-91)	77	0(0-41)	83(73-91)	76
DGM	40(16-68)	94(86-98)	84	33(4-78)	90(81-95)	86	43(10-82)	91(82-96)	87
Cerebellum	13(2-40)	93(84-98)	78	17(1-64)	92(84-97)	87	29(4-71)	93(85-98)	88
Global Score	33(12-62)	87(76-94)	77	50(12-88)	86(76-93)	83	43(10-82)	86(76-93)	82
Term MRI (n=77)									
WM	14(2-43)	92(82-97)	78	33(4-78)	93(84-98)	88	29(4-71)	93(84-98)	87
CGM	21(5-51)	79(67-89)	69	0(0-46)	77(66-87)	71	29(4-71)	80(69-89)	75
DGM	36(13-65)	86(75-93)	77	33(4-78)	83(72-91)	79	71(29-96)	87(77-94)	86
Cerebellum	21(5-51)	92(82-97)	79	33(4-78)	92(83-97)	87	43(10-82)	93(84-98)	88
Global Score	14(2-43)	90(80-96)	77	33(4-78)	92(83-97)	87	29(4-71)	91(82-97)	86

Key: Sensitivity and specificity: percentage (95%CI); Correctly classified: percentage; Early MRI, 29-35 weeks PMA; Term MRI, 40-42 weeks PMA; CGM cortical gray matter; DGM deep gray matter

On-line Table 1: Scoring parameters and findings in preterm sample Early and Term MRI, and term reference sample MRI, presented as number (%)

	Score		Early MRI n=83 (t1)	Term MRI n=77 (t2)	Reference n=38	Difference t1-t2 (*p*)
Cerebral WM	0	None	76(92%)	73(95%)	38(100%)	0.71
Cystic lesions	1	Focal unilateral	5(6%)	2(3%)	0	
	2	Focal bilateral	1(1%)	1(1%)	0	
	3	Extensive unilateral	0	0	0	
	4	Extensive bilateral	1(1%)	1(1%)	0	
Focal signal abnormality	0	None	62(75%)	66(86%)	38(100%)	0.05
	1	Focal punctate	12(14%)	4(5%)	0	
	2	Extensive punctate	2(2%)	2(3%)	0	
	3	Linear	7(8%)	5(6%)	0	
Myelination delay	0	Myelinated PLIC & corona radiata	0	72(94%)	38(100%)	<0.01
	1	Only PLIC myelinated	0	1(1%)	0	
	2	Minimal myelination–no myelin in PLIC	83(100%)#	4(5%)	0	
Thinning of Corpus Callosum	0	Genu, midbody, & splenium <2SD below mean	80(96%)	61(79%)	37(97%)	<0.01
	1	Genu or midbody or splenium >2SD below mean	1(1%)	14(18%)	1(3%)	
	2	Genu or midbody & splenium >2SD below mean	2(2%)	2(3%)	0	
Dilated lateral ventricles	0	Both sides within 2 SD of mean	69(83%)	55(71%)	36(95%)	0.24
	1	One side >2SD but <3SD above mean	6(7%)	15(19%)	2(5%)	
	2	One or both sides >3SD above mean	8(10%)	7(9%)	0	
Volume reduction	0	cBPW <2SD below mean	34(41%)	31(40%)	37(97%)	0.51
	1	cBPW >2SD below but <3SD below mean	29(35%)	27(35%)	1(3%)	
	2	cBPW >3SD below mean	20(24%)	19(25%)	0	
Cortical GM	0	None	83(100%)	77(99%)	38(100%)	0.32
Signal abnormality	1	Focal unilateral	0	0	0	
	2	Focal bilateral	0	1(1%)	0	
	3	Extensive unilateral	0	0	0	
	4	Extensive bilateral	0	0	0	
Gyral maturation	0	Delay <2 weeks	63(76%)	75(97%)	38(100%)	<0.01
	1	2 ≤ delay < 4 weeks	13(16%)	2(3%)	0	
	2	Delay ≥ 4 weeks	7(8%)	0	0	

Increased extracerebral	0	cIHD within 2 SD of mean	68(82%)	55(71%)	37(97%)	0.06
space	1	cIHD >2SD & <3SD above mean	10(12%)	7(9%)	1(3%)	
	2	cIHD >3SD above mean	5(6%)	15(19%)	0	
Deep GM	0	None	75(90%)	75(97%)	38(100%)	0.01
Signal abnormality	1	Focal unilateral	5(6%)	1(1%)	0	
	2	Focal bilateral	3(4%)	1(1%)	0	
	3	Extensive unilateral	0	0	0	
	4	Extensive bilateral	0	0	0	
Volume reduction	0	cDGMA <2SD below mean	59(71%)	50(64%)	37(97%)	0.06
	1	cDGMA >2SD below & <3SD below mean	17(20%)	13(17%)	1(3%)	
	2	cDGMA >3SD below mean	7(8%)	14(18%)	0	
Cerebellum	0	None	78(94%)	72(94%)	38(100%)	0.32
Signal abnormality	1	Punctate unilateral	4(5%)	5(6%)	0	
	2	Punctate bilateral	0	0	0	
	3	Extensive unilateral	1(1%)	0	0	
	4	Extensive bilateral	0	0	0	
Volume reduction	0	cTCD <2SD below mean	69(83%)	54(70%)	37(97%)	0.09
	1	cTCD >2SD below & <3SD below mean	8(10%)	16(21%)	1(3%)	
	2	cTCD >3SD below mean	6(7%)	7(9%)	0	

Key: Early MRI, 29-35 weeks PMA; Term MRI, 40-42 weeks PMA; Mean and SD refer to term reference sample data; GM gray matter; cBPW corrected biparietal width; cDGMA corrected deep GM area; cTCD corrected transcerebellar diameter; IHD interhemispheric distance; PLIC posterior limb of the internal capsule; VD ventricular diameter; # all Early MRIs scored 2 for myelination delay to represent 'unmyelinated PLIC and corona radiata'. $p<0.05$ represents a significant difference between Early and Term MRI scores in the preterm sample.

On-line Table 2: Structural MRI scoring system for use from 29-46 weeks postmenstrual age in preterm infants – Score Sheet

Date:
Patient ID:
Postmenstrual age (PMA) at MRI: 'c' denotes corrected value

Cerebral WM	Score 0	Score 1	Score 2	Score 3	Score 4	Comments
Cystic lesions	None	Focal unilateral (On-line Fig 1)	Focal bilateral (On-line Fig 2)	Extensive unilateral (On-line Fig 3)	Extensive bilateral (On-line Fig 4)	
Focal signal abnormality	None	Focal punctate (On-line Fig 5)	Extensive punctate (On-line Fig 6)	Linear (On-line Fig 7)		
Myelination delay	Myelinated PLIC & corona radiata	Only PLIC myelinated	Minimal myelination – no myelin in PLIC			
Thinning of corpus callosum	Measure genu, midbody & splenium on a midsagittal section & correct genu & splenium for PMA at MRI using equations: cGenu = measured genu + 0.03x(40-PMA at MRI) cSplenium = measured splenium + 0.03x(40-PMA at MRI)					
(On-line Fig 8)	cGenu >1.13mm & Midbody >0.7mm & cSplenium >1.84mm	cGenu <1.13mm OR Midbody <0.7mm OR cSplenium <1.84mm	(cGenu <1.13mm OR Midbody <0.7mm) & cSplenium <1.84mm			
Dilated lateral ventricles	Measure left (LV) & right ventricle (RV) at level of ventricular atrium & correct for PMA at MRI using equation: cRV = measured RV + 0.15x(40-PMA at MRI) cLV = measured LV + 0.13x(40-PMA at MRI)					
(On-line Fig 9)	cRV<9.12mm & cLV<8.42mm	One or both: 9.12mm<cRV<10.39mm 8.42mm<cLV<9.39mm	One or both: cRV>10.39mm cLV>9.39mm			
Volume reduction	Measure biparietal width (BPW) at level of the basilar turn of the cochlea & correct for PMA at MRI using equation: Corrected BPW= measured BPW + 2.33x(40-PMA at MRI)					
(On-line Fig 10)	cBPW >78.52mm	74.6mm <cBPW< 78.52mm	cBPW <74.6mm			

WM total = /15

Cortical GM	Score 0	Score 1	Score 2	Score 3	Score 4	Comments
Signal abnormality	None	Focal unilateral	Focal bilateral	Extensive unilateral	Extensive bilateral	
Gyral maturation	34-36weeks PMA, marginal sulcus & paracentral gyrus present; secondary sulci in frontal lobes, superior & middle temporal & prerolandic, postrolandic, insula, & occipital regions present. 36-38weeks PMA, additional secondary gyri in transverse & inferior temporal; anterior & posterior orbital gyri established. 40weeks PMA, tertiary inferior temporal & inferior occipital gyri & sulci (Inder *et al* 2003).					
Increased extracerebral space (On-line Fig 10)	Delay <2 weeks Measure interhemispheric distance (IHD) between crowns of superior frontal gyri at same section as measurement for BPW. Correct for PMA at MRI using equation: cIHD = measured IHD + 0.16x(40- PMA at MRI) cIHD <3.98mm	2 ≤ delay < 4 weeks 3.98mm <cIHD< 4.69mm	Delay ≥ 4 weeks cIHD >4.69mm			Cortical GM total= /8

Deep GM	Score 0	Score 1	Score 2	Score 3	Score 4	Comments
Signal abnormality	None	Focal unilateral (On-line Fig 11)	Focal bilateral (On-line Fig 12)	Extensive unilateral (On-line Fig 13)	Extensive bilateral (On-line Fig 14)	
Volume reduction (On-line Fig 15)	Measure deep gray matter area (DGMA) on a single axial section where caudate heads, lentiform nuclei, and thalami are maximally visible. Correct for PMA at MRI using equation: cDGMA = measured DGMA + 0.45x(40- PMA at MRI) cDGMA >11.1mm	10.51mm <cDGMA< 11.1mm	cDGMA <10.51mm			Deep GM total = /6

Cerebellum	Score 0	Score 1	Score 2	Score 3	Score 4	Comments
Signal abnormality	None	Punctate unilateral (On-line Fig 16)	Punctate bilateral (On-line Fig 17)	Extensive unilateral (On-line Fig 18)	Extensive bilateral	
Volume reduction (On-line Fig 9)	Measure transcerebellar diameter (TCD) at level of atria, maximal horizontal distance. Correct for PMA at MRI using equation: cTCD = measured TCD + 1.78x(40- PMA at MRI) cTCD >50.02mm	48.04mm <cTCD<50.02mm	cTCD <48.04mm			Cerebellum total = /6

Global total = /35 **Global score category** (0-3 normal; 4-7 mild; 8-11 moderate; 12+ severe)

On-line Table 3: Relationship between postmenstrual age at MRI and each regional measurement

	Preterm sample n=135 (n=71 Early MRI, n=64 Term MRI)			**Term reference sample n=38**		
	Regression Coefficient	**95% CI**	***p***	**Regression Coefficient**	**95% CI**	***p***
Corpus callosum genu	0.03	0.01; 0.05	**0.01**	0.28	0.02; 0.53	**0.03**
Corpus callosum body	0.01	-0.01; 0.02	0.33	-0.03	-0.19; 0.12	0.64
Corpus callosum splenium	0.03	0.01; 0.04	**<0.01**	0.04	-0.15; 0.23	0.67
Ventricular diameter right	0.15	0.11; 0.18	**<0.01**	0.35	-0.06; 0.76	0.09
Ventricular diameter left	0.13	0.1; 0.16	**<0.01**	0.10	-0.22; 0.43	0.51
Biparietal width	2.33	2.19; 2.48	**<0.01**	0.00	-1.32; 1.33	0.99
Interhemispheric distance	0.16	0.12; 0.2	**<0.01**	0.03	-0.21; 0.27	0.78
Deep gray matter area	0.45	0.42; 0.47	**<0.01**	0.11	-0.09; 0.3	0.27
Transcerebellar diameter	1.78	1.72; 1.84	**<0.01**	0.99	0.33; 1.66	**0.01**

Key: Early MRI, 29-35 weeks PMA; Term MRI, 40-42 weeks PMA; Preterm sample- cases with focal brain lesions were removed to ensure that any linear relationship found was the result of age and not confounded by brain injury. Regression analyses were conducted separately for the preterm and term samples.

On-line Table 4: Corrected means and standard deviations of the regional measurements for Early and Term MRI for the preterm group, and the term reference sample[a].

	Early MRI n=83	**Term MRI n=77**	**Term reference sample n=38**
Corpus callosum genu	1.96 (0.43)	1.91 (0.67)	2.63 (0.75)
Corpus callosum body	1.33 (0.28)[b]	1.40 (0.43)[b]	1.60 (0.45) [b]
Corpus callosum splenium	2.71 (0.51)	2.61 (0.62)	2.98 (0.57) [b]
Ventricular diameter right	7.43 (3.29)	7.49 (3.58)	6.48 (0.97) [b]
Ventricular diameter left	7.51 (3.20)	7.70 (4)	6.58 (1.3) [b]
Biparietal width	77.40 (4.47)	77.40 (5.44)	86.38 (3.93) [b]
Interhemispheric distance	3.27 (0.79)	3.50 (1.33)	2.56 (0.71) [b]
Deep gray matter area	11.48 (0.82)	11.44 (1.01)	12.28 (0.59) [b]
Transcerebellar diameter	51.55 (2.53)	51.34 (2.73)	53.98 (1.98)

Key: [a] For each measure that demonstrated a linear relationship with PMA at MRI in On-line Table 3, the regression coefficient (slope) was used to generate an equation for correction, written as: Corrected Value = Measured Value + Regression Coefficient x (40-PMA at MRI). A single equation was used to correct Early and Term MRI regional measures for the preterm group. The term reference sample data were corrected separately.

[b] Uncorrected values—that is, for regional measures in which no linear relationship was found and no correction was performed.

On-line Table 5: Inter- and intrarater reproducibility of Early and Term MRI scores

	Inter-rater (n=20)		Intra-rater (n=20)	
Early MRI scores	**Reliability ICC [95% CI]**	**% Agreement**	**Reliability ICC [95% CI]**	**% Agreement**
White matter	0.79 [0.47, 0.92]	70	0.97 [0.93, 0.99]	95
CGM	0.08 [0.00, 0.63]	95	0.92 [0.80, 0.97]	100
DGM	0.86 [0.64, 0.94]	85	0.92 [0.81, 0.97]	90
Cerebellum	0.76 [0.40, 0.91]	95	0.82 [0.51, 0.93]	95
Global	0.89 [0.72, 0.96]	80	0.97 [0.91, 0.99]	95
Term MRI scores				
White matter	0.93 [0.82, 0.97]	90	0.96 [0.89, 0.98]	90
CGM	0.66 [0.15, 0.87]	90	0.75 [0.32, 0.91]	95
DGM	0.86 [0.66, 0.95]	90	0.96 [0.89, 0.98]	100
Cerebellum	0.91 [0.78, 0.97]	100	0.91 [0.77, 0.97]	100
Global	0.93 [0.82, 0.97]	80	0.97 [0.92, 0.99]	95

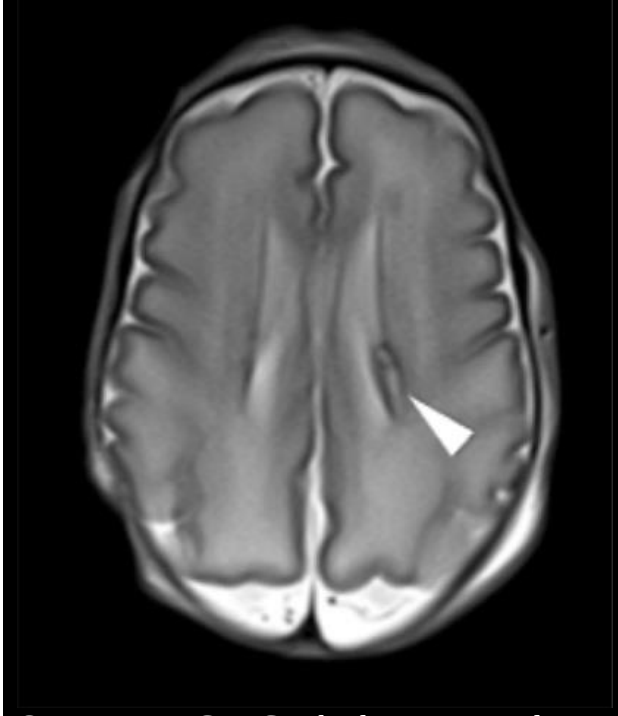
Key: "% agreement" is defined as an exact agreement ±1 point for subscale scores and exact agreement ±2 points for global scores; CGM cortical gray matter; DGM deep gray matter.

On-line Table 6: Perinatal variables and grade of global brain abnormality of infants included in this validation paper

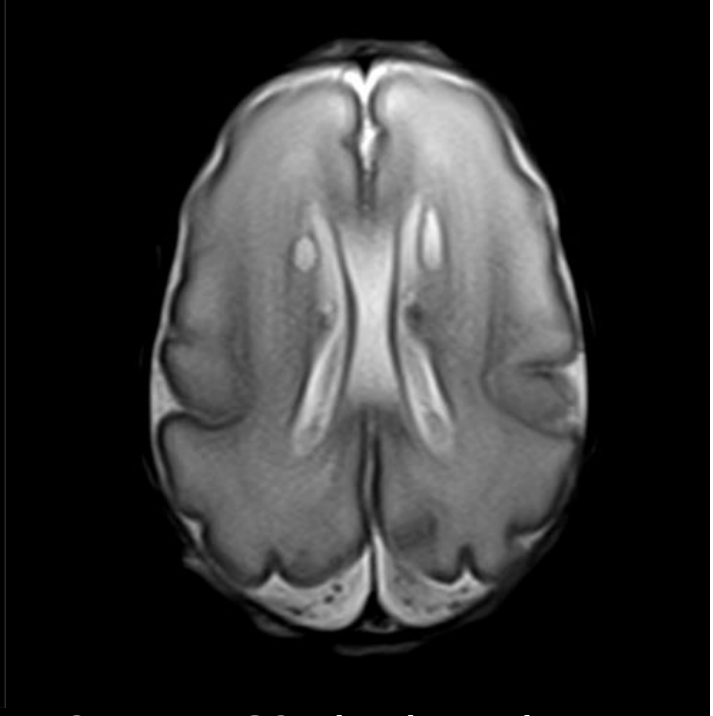
Variables	Global Brain Abnormality								*p* value	
	Normal (Score 0-3)		Mild (Score 4-7)		Moderate (Score 8-11)		Severe (Score ≥12)			
	Early MRI n = 28	Term MRI n = 49	Early MRI n = 41	Term MRI n = 20	Early MRI n = 10	Term MRI n = 5	Early MRI n = 4	Term MRI n = 3	Early MRI	Term MRI
GA at birth (weeks)	28^{+6} (1^{+5})	28^{+3} (1^{+6})	27^{+6} (1^{+6})	28^{+2} (1^{+3})	27^{+3} (2^{+0})	25^{+3} (1^{+0})	27^{+0} (2^{+4})	27^{+3} (2^{+6})	**0.04**	**0.03**
Birth weight (g)	1213 (266)	1145 (318)	1018 (305)	1001 (280)	913 (306)	820 (94)	974 (427)	878 (587)	**<0.01**	**0.04**
Male sex	17 (61%)	30 (61%)	24 (59%)	12 (60%)	5 (50%)	3 (60%)	3 (75%)	1 (33%)	0.85	0.64
Multiple births	8 (29%)	12 (25%)	12 (29%)	7 (35%)	4 (40%)	2 (40%)	0 (0%)	0 (0%)	0.95	0.58
Maternal PROM	10 (36%)	14 (29%)	7 (17%)	2 (10%)	1 (10%)	1 (20%)	1 (25%)	1 (33%)	0.07	0.23
Chorioamnionitis	8 (29%)	9 (18%)	5 (12%)	3 (15%)	0 (0%)	0 (0%)	1 (25%)	1 (33%)	0.05	0.66
Antenatal steroids	23 (82%)	36 (74%)	29 (71%)	17 (85%)	8 (80%)	1 (20%)	2 (50%)	3 (100%)	0.30	0.88
Higher social risk	11 (41%)	16 (33%)	19 (46%)	12 (60%)	7 (70%)	4 (80%)	2 (50%)	2 (67%)	0.21	**<0.01**
PDA	7 (25%)	21 (43%)	24 (59%)	8 (40%)	5 (50%)	5 (100%)	3 (75%)	2 (67%)	**0.01**	0.19
Maternal $MgSO_4$	16 (64%)	26 (59%)	20 (63%)	12 (75%)	6 (86%)	2 (100%)	1 (50%)	1 (100%)	0.67	0.11
Caesarian section	20 (71%)	34 (69%)	30 (73%)	15 (75%)	7 (70%)	4 (80%)	3 (75%)	3 (100%)	0.95	0.32
ROP	7 (25%)	14 (29%)	20 (49%)	13 (65%)	8 (80%)	4 (80%)	3 (75%)	2 (67%)	**<0.01**	**<0.01**
NEC	1 (4%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (50%)	1 (33%)	0.22	0.50
Confirmed sepsis	2 (7%)	2 (4%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.26	0.41
TPN (days)	10 [6-13]	11 [8-14]	12 [8-15]	11 [7-14]	13 [8-14]	14 [14-19]	18 [6-28]	11 [n.d.]	0.12	0.17
Postnatal steroids	1 (4%)	7 (14%)	7 (17%)	3 (15%)	3 (30%)	2 (40%)	3 (75%)	2 (67%)	**<0.01**	0.11
Ventilation (days)	2 [0-3]	2 [0-8]	3 [2-18]	3 [0-7]	3 [2-34]	30 [11-42]	31 [8-35]	20 [n.d.]	**0.02**	**0.04**
CPAP (days)	12 [4-21]	18 [6-46]	17 [8-32]	33 [9-52]	15 [10-27]	50 [37-55]	7 [3-23]	32 [n.d.]	0.14	0.12
Oxygen therapy (hours)	5 [1-42]	46 [2-386]	51 [5-253]	6 [1-62]	1 [1-774]	512 [n.d.]	665 [114-1358]	1958 [n.d.]	**0.01**	0.57
BPD	n.a.	11 (22%)	n.a.	6 (30%)	n.a.	4 (80%)	n.a.	2 (67%)	n.a.	**0.02**
Home oxygen	n.a.	5 (100%)	n.a.	4 (20%)	n.a.	3 (60%)	n.a.	1 (33%)	n.a.	**0.02**

Key: Continuous measures reported as mean (SD) or median [IQR]. Categorical measures reported as frequency (percentage). BPD bronchopulmonary dysplasia (oxygen requirement at 36 weeks PMA); CPAP continuous positive airway pressure; NEC necrotizing enterocolitis (diagnosed or suspected); PDA patent ductus arteriosus; PROM premature rupture of membranes; ROP retinopathy of prematurity; TPN total parenteral nutrition. n.a., not applicable; n.d., not determinable due to small sample size; *p* value<0.05 indicates a significant association between the perinatal variable and increasing severity of MRI global brain abnormality category score

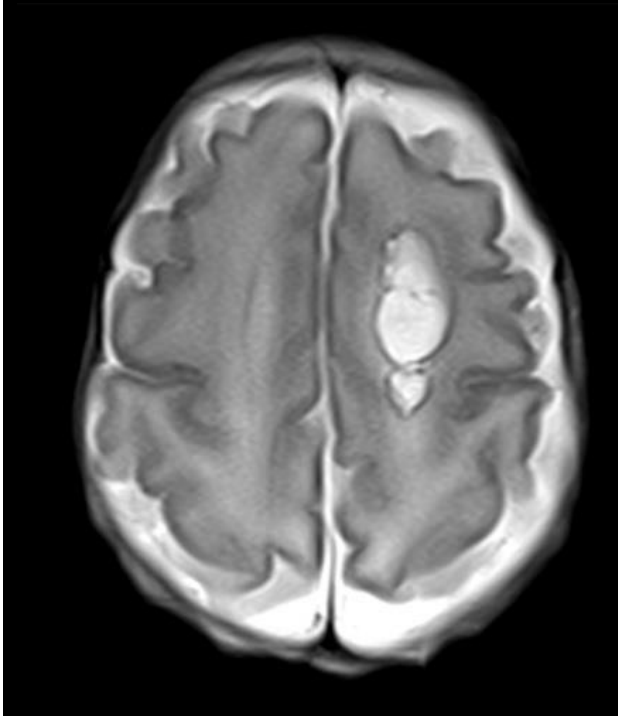
Online Figures



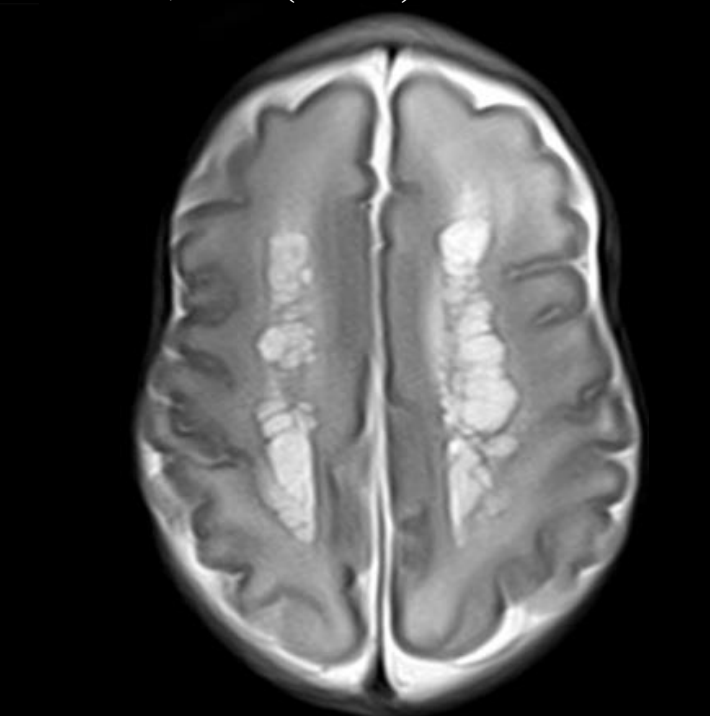
ON-LINE FIG 1. Cerebral WM, cystic lesion, focal unilateral, score 1 (axial T2).



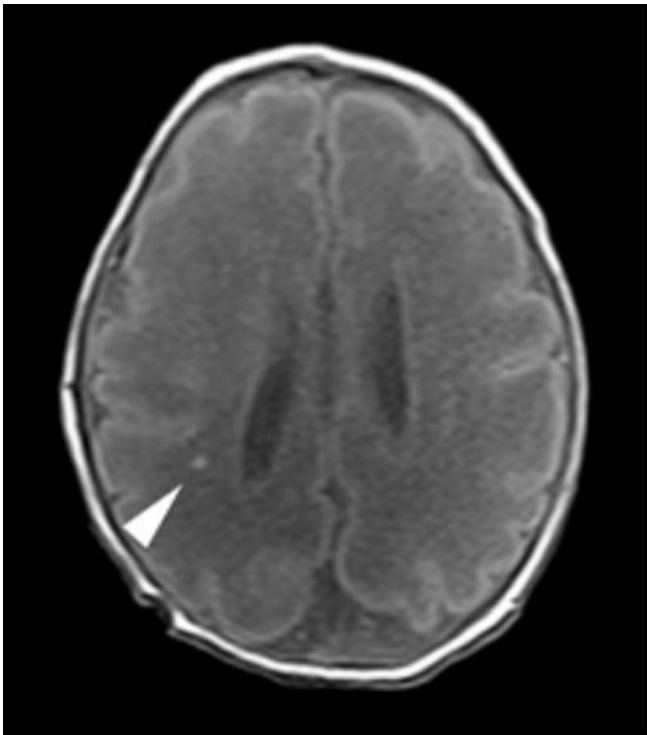
ON-LINE FIG 2. Bilateral connatal cysts, classified as cerebral WM, cystic lesion, focal bilateral, score 2 (axial T2).



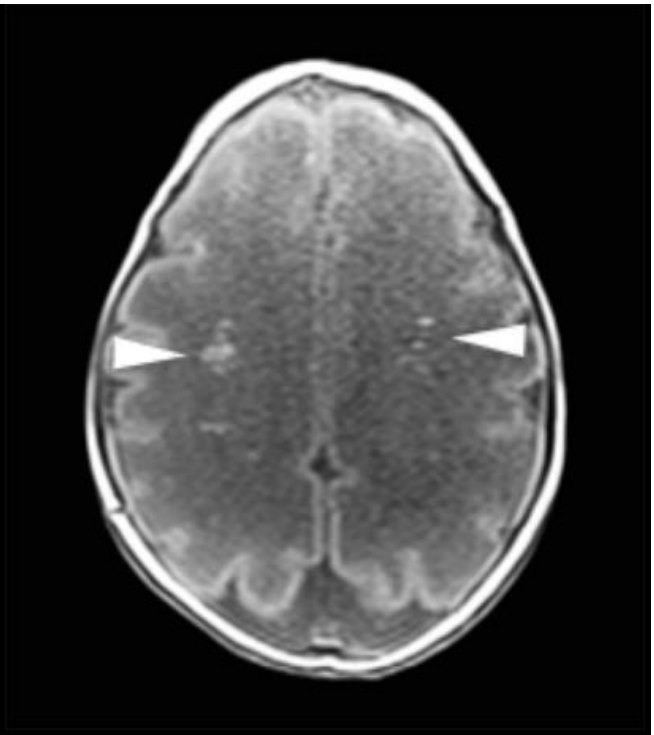
ON-LINE FIG 3. Cerebral WM, cystic lesion, extensive unilateral, score 3 (axial T2).



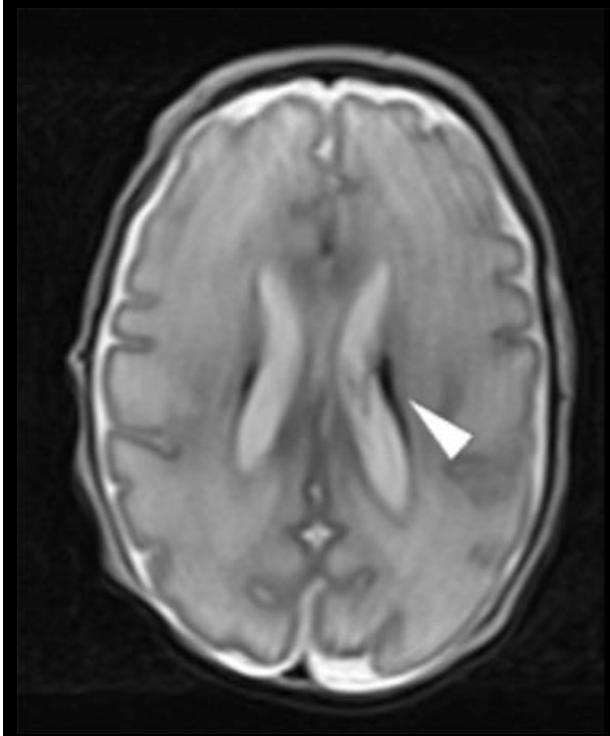
ON-LINE FIG 4. Cerebral WM, cystic lesion, extensive bilateral, score 4 (axial T2).



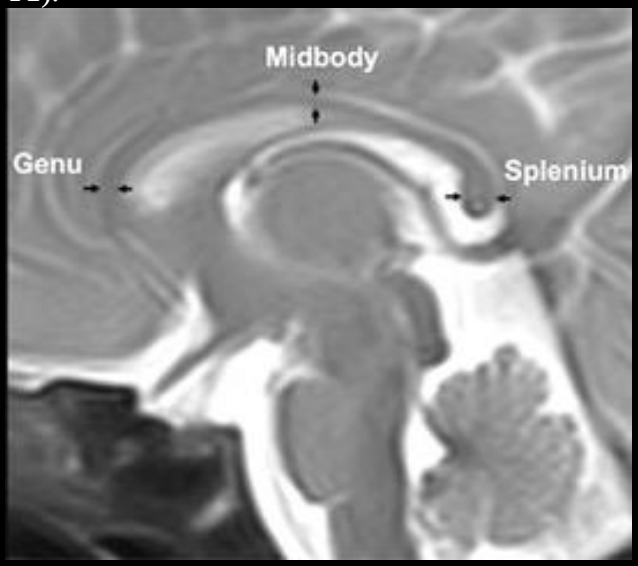
ON-LINE FIG 5. Cerebral WM, focal signal abnormality, focal punctate, score 1 (axial T1).



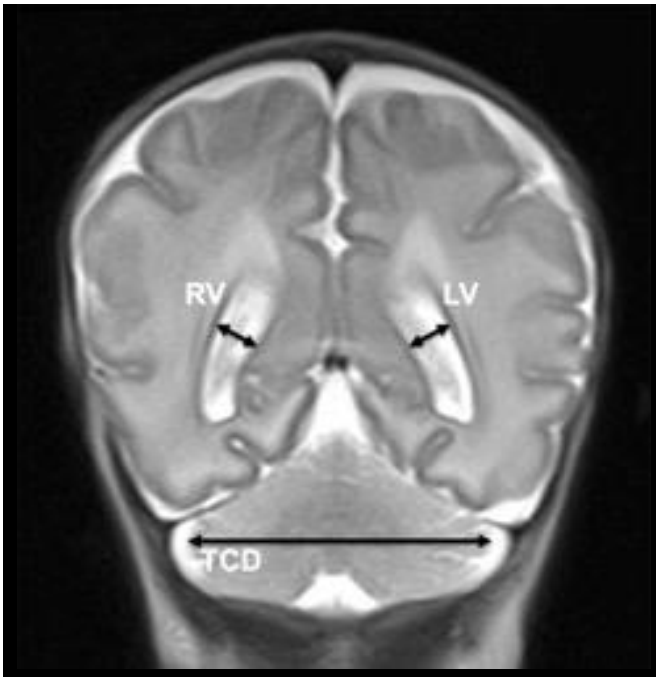
ON-LINE FIG 6. Cerebral WM, focal signal abnormality, extensive punctate, score 2 (axial T1).



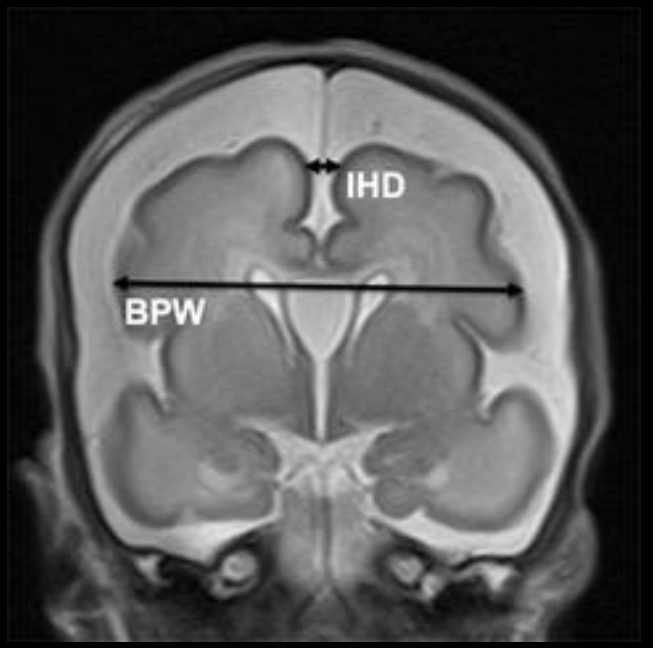
ON-LINE FIG 7. Cerebral WM, focal signal abnormality, linear, score 3 (axial T2).



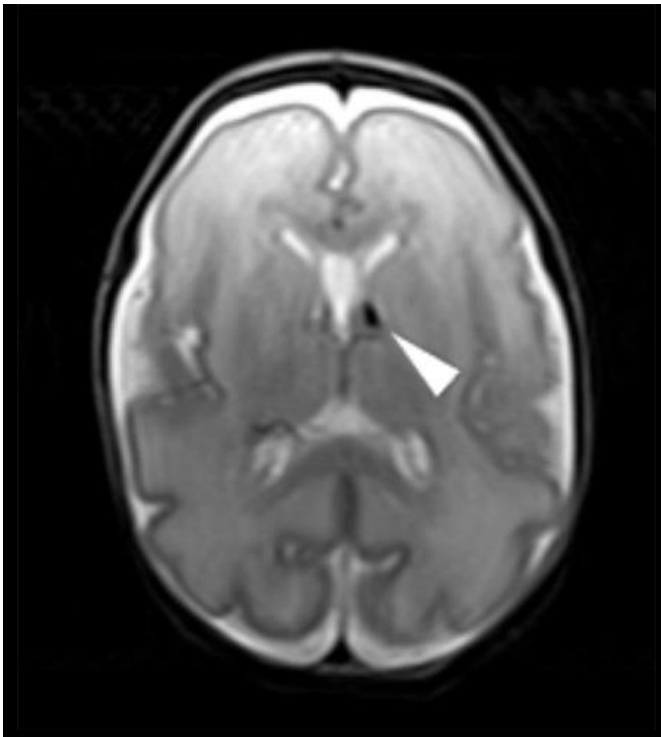
ON-LINE FIG 8. Cerebral WM, thinning of the corpus callosum. Measure genu, midbody, and splenium on a midsaggital section (T2) and correct genu and splenium for PMA at MR imaging by using the following equations: cGenu = Measured Genu + 0.03 × (40-PMA at MRI). cSplenium = Measured Splenium +0.03 × (40-PMA at MRI). Score 0: cGenu > 1.13 mm and midbody > 0.7 mm and cSplenium > 1.84 mm. Score 1: cGenu < 1.13 mm or midbody < 0.7 mm or cSplenium < 1.84 mm. Score 2: (cGenu <1.13 mm or midbody < 0.7 mm) and cSplenium <1.84 mm.



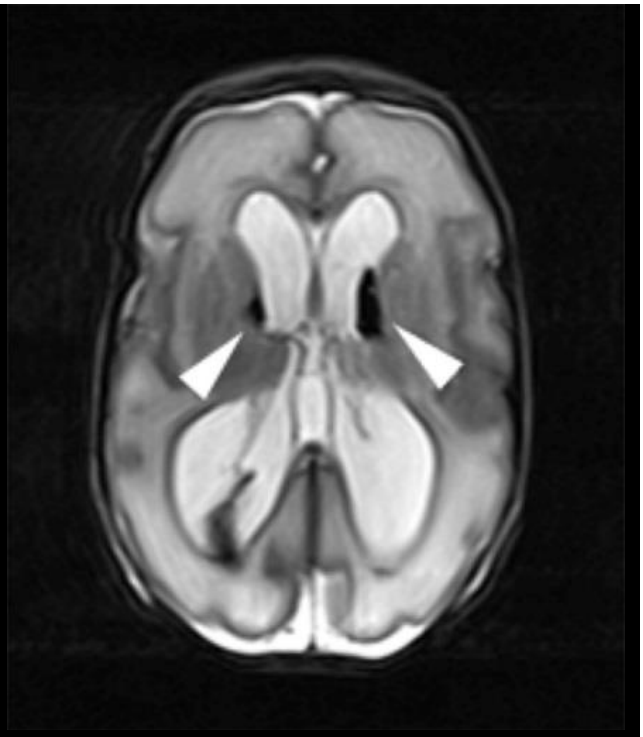
ON-LINE FIG 9. Cerebral WM, dilated lateral ventricles. Measure left (LV) and right ventricle (RV) at the level of the ventricular atrium (coronal T2) and correct for PMA at MRimaging by using the following equation: cRV = Measured RV + 0.15 × (40-PMA at MRI). cLV = Measured LV+ 0.13 × (40-PMA at MRI). Score 0: cRV < 9.12 mm and cLV<8.42 mm. Score 1: One or both: 9.12 mm<cRV<10.39 mm; 8.42 mm< cLV < 9.39 mm. Score 2: One or both: cRV> 10.39 mm; cLV> 9.39 mm. Cerebellum, volume reduction. Measure transcerebellar diameter (TCD) at the level of the atria, maximal horizontal distance (coronal T2), correct for PMA at MR imaging by using the following equation: cTCD= Measured TCD + 1.78×(40-PMA at MRI). Score 0: cTCD > 50.02 mm. Score 1: 48.04 mm<cTCD < 50.02 mm. Score 2: cTCD< 48.04 mm.



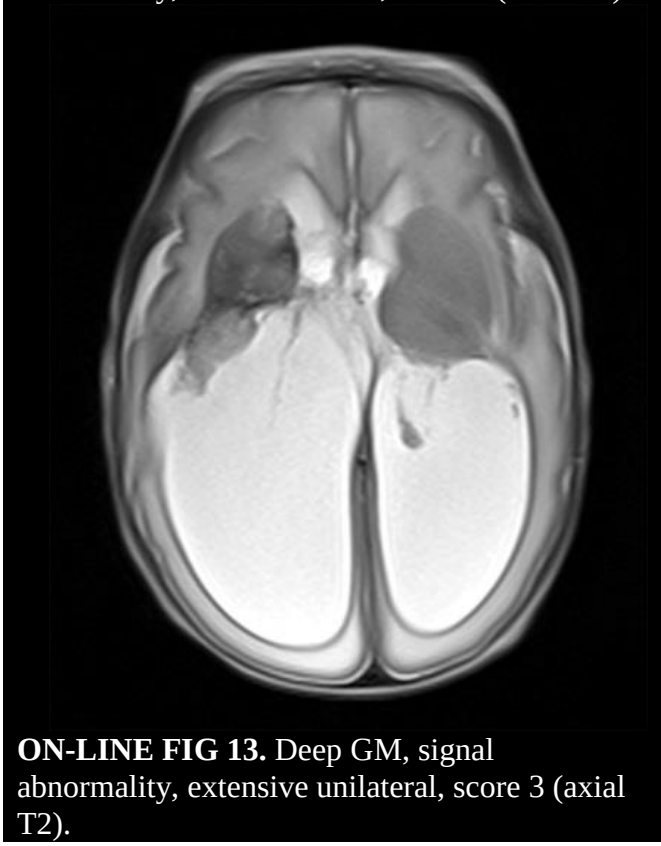
ON-LINE FIG 10. CerebralWM,volume reduction. Measure biparietal width at level of the basilar turn of the cochlea (coronal T2) and correct for PMA at MR imaging by using the following equation: Corrected BPW= Measured BPW + 2.33×(40-PMA at MRI). Score 0: cBPW> 78.52 mm. Score 1: 74.6 mm< cBPW < 78.52 mm. Score 2: cBPW < 74.6 mm. Cortical GM, increased extracerebral space (coronal T2). Measure the interhemispheric distance (IHD) between the crowns of the superior frontal gyri at the same section as measurement for BPW. Correct for PMA at MRimaging by using the following equation: cIHD = Measured IHD + 0.16 × (40-PMA at MRI). Score 0: cIHD < 3.98 mm. Score 1: 3.98 mm < cIHD < 4.69 mm. Score 2: cIHD> 4.69 mm.



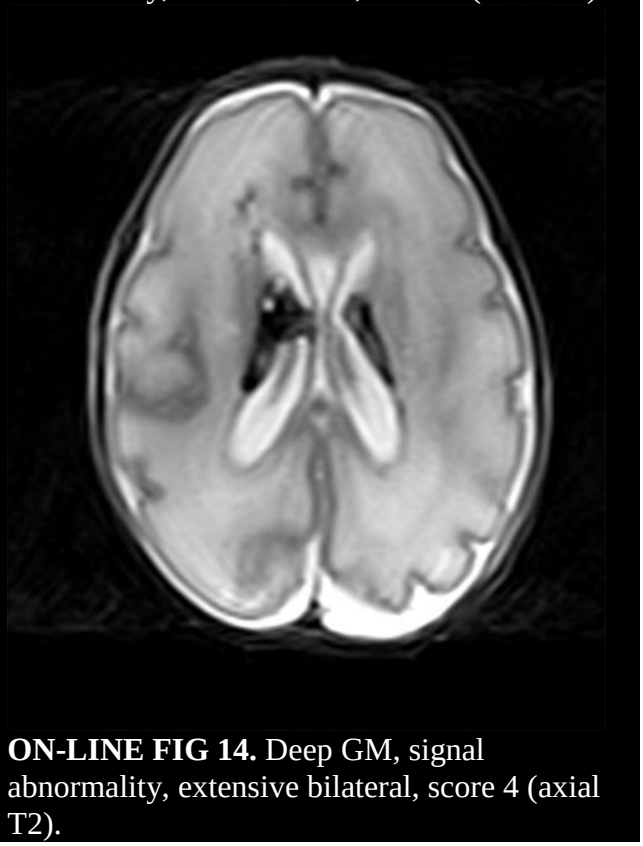
ON-LINE FIG 11. Deep GM, signal abnormality, focal unilateral, score 1 (axial T2).



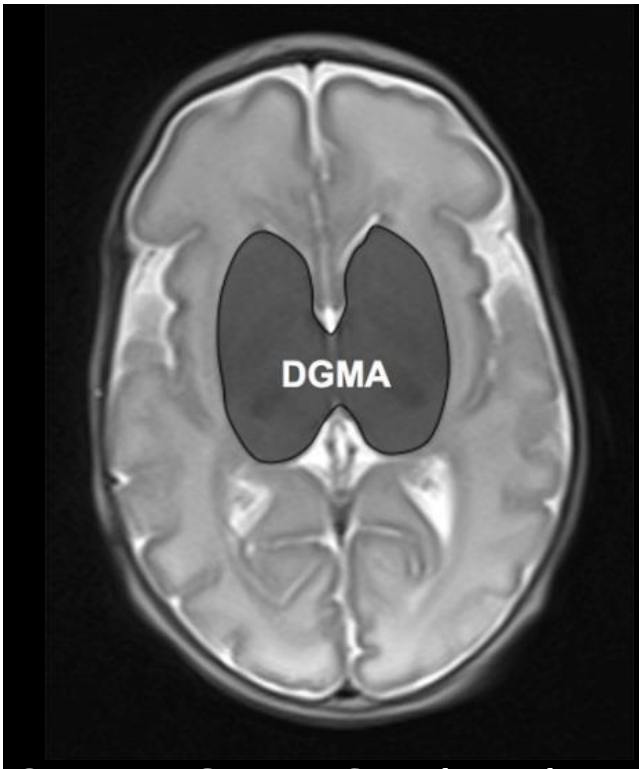
ON-LINE FIG 12. Deep GM, signal abnormality, focal bilateral, score 2 (axial T2).



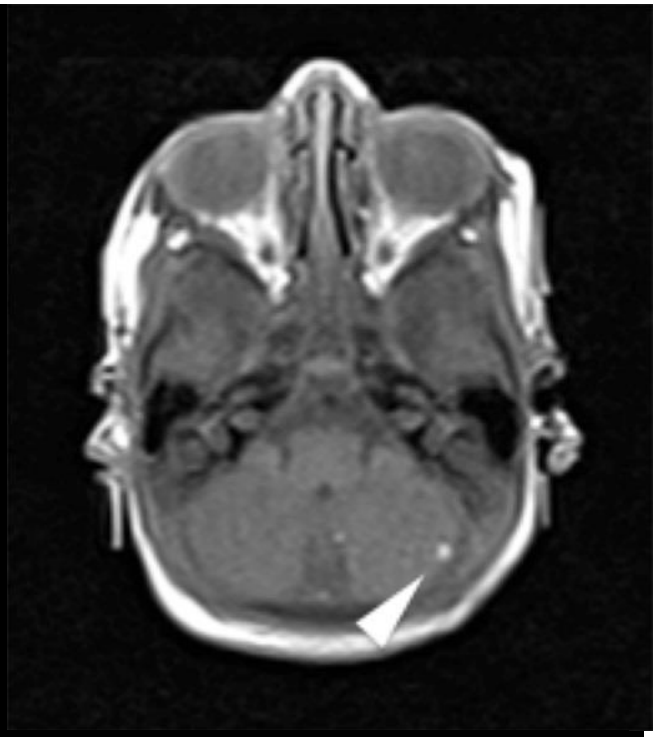
ON-LINE FIG 13. Deep GM, signal abnormality, extensive unilateral, score 3 (axial T2).



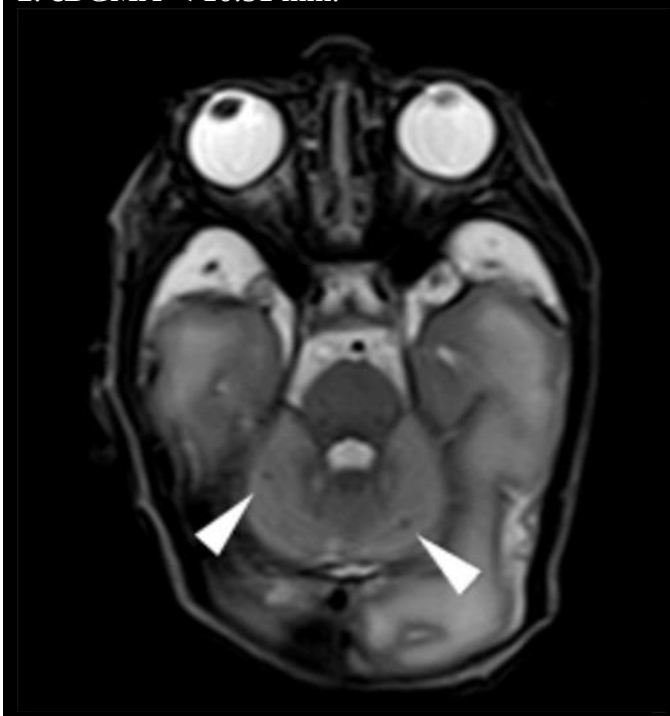
ON-LINE FIG 14. Deep GM, signal abnormality, extensive bilateral, score 4 (axial T2).



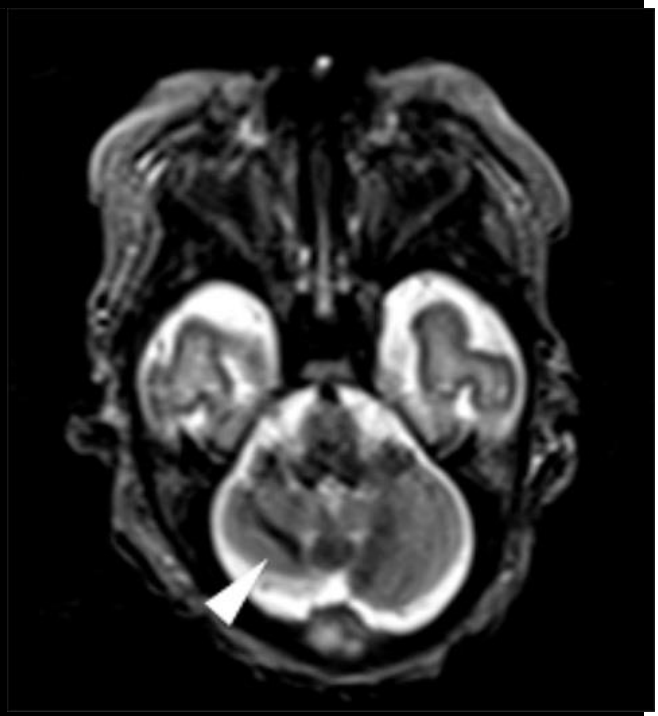
ON-LINE FIG 15. DeepGM, volume reduction. Measure deep gray matter area (DGMA) on a single axial section where the caudate heads, lentiform nuclei, andthalami are maximally visible (axial T2). Correct forPMA at MR imaging by using the following equation: cDGMA = Measured DGMA + 0.45 × (40-PMA at MRI). Score 0: cDGMA > 11.1 mm. Score 1: 10.51 mm< cDGMA <11.1 mm. Score 2: cDGMA < 10.51 mm.



ON-LINE FIG 16. Cerebellum, signal abnormality, punctate unilateral, score 1 (axial T1).



ON-LINE FIG 17. Cerebellum, signal abnormality, punctate bilateral, score 2 (axial T2).



ON-LINE FIG 18. Cerebellum, signal abnormality, extensive unilateral, score 3 (axial T2).

References

1. Woodward LJ, Anderson PJ, Austin NC, Howard K, Inder TE. Neonatal MRI to predict neurodevelopmental outcomes in preterm infants. *N Engl J Med* 2006; **355**: 685-94.

2. Woodward LJ, Clark CA, Bora S, Inder TE. Neonatal white matter abnormalities an important predictor of neurocognitive outcome for very preterm children. *PLoS One* 2012; **7**: e51879.

3. Kidokoro H, Anderson PJ, Doyle LW, Woodward LJ, Neil JJ, Inder TE. Brain injury and altered brain growth in preterm infants: predictors and prognosis. *Pediatrics* 2014; **134**: e444-53.

4. Spittle AJ, Boyd RN, Inder TE, Doyle LW. Predicting motor development in very preterm infants at 12 months' corrected age: the role of qualitative magnetic resonance imaging and general movements assessments. *Pediatrics* 2009; **123**: 512-7.

5. Spittle AJ, Cheong J, Doyle LW, et al. Neonatal white matter abnormality predicts childhood motor impairment in very preterm children. *Dev Med Child Neurol* 2011; **53**: 1000-6.

6. Volpe JJ. The encephalopathy of prematurity--brain injury and impaired brain development inextricably intertwined. *Semin Pediatr Neurol* 2009; **16**: 167-78.

7. Miller SP, Ferriero DM, Leonard C, et al. Early brain injury in premature newborns detected with magnetic resonance imaging is associated with adverse early neurodevelopmental outcome. *J Pediatr* 2005; **147**: 609-16.

8. Inder TE, Wells SJ, Mogridge NB, Spencer C, Volpe JJ. Defining the nature of the cerebral abnormalities in the premature infant: a qualitative magnetic resonance imaging study. *J Pediatr* 2003; **143**: 171-9.

9. Sie LT, Hart AA, van Hof J, et al. Predictive value of neonatal MRI with respect to late MRI findings and clinical outcome. A study in infants with periventricular densities on neonatal ultrasound. *Neuropediatrics* 2005; **36**: 78-89.

10. Spittle AJ, Brown NC, Doyle LW, et al. Quality of general movements is related to white matter pathology in very preterm infants. *Pediatrics* 2008; **121**: e1184-9.

11. Peyton C, Yang E, Kocherginsky M, et al. Relationship between white matter pathology and performance on the General Movement Assessment and the Test of Infant Motor Performance in very preterm infants. *Early Hum Dev* 2016; **95**: 23-7.

12. Brown NC, Inder TE, Bear MJ, Hunt RW, Anderson PJ, Doyle LW. Neurobehavior at term and white and gray matter abnormalities in very preterm infants. *J Pediatr* 2009; **155**: 32-8, 8 e1.

13. Pineda RG, Neil J, Dierker D, et al. Alterations in brain structure and neurodevelopmental outcome in preterm infants hospitalized in different neonatal intensive care unit environments. *J Pediatr* 2014; **164**: 52-60 e2.

14. Iwata S, Nakamura T, Hizume E, et al. Qualitative brain MRI at term and cognitive outcomes at 9 years after very preterm birth. *Pediatrics* 2012; **129**: e1138-47.

15. Hintz SR, Barnes PD, Bulas D, et al. Neuroimaging and neurodevelopmental outcome in extremely preterm infants. *Pediatrics* 2015; **135**: e32-42.

16. Skiold B, Vollmer B, Bohm B, et al. Neonatal magnetic resonance imaging and outcome at age 30 months in extremely preterm infants. *J Pediatr* 2012; **160**: 559-66.e1.

17. Tich SNT, Anderson PJ, Shimony JS, Hunt RW, Doyle LW, Inder TE. A Novel Quantitative Simple Brain Metric Using MR Imaging for Preterm Infants. *AJNR Am J Neuroradiol* 2009; **30**: 125-31.

18. Spittle AJ, Doyle LW, Anderson PJ, et al. Reduced cerebellar diameter in very preterm infants with abnormal general movements. *Early Hum Dev* 2010; **86**: 1-5.

19. Melbourne L, Murnick J, Chang T, Glass P, Massaro AN. Regional Brain Biometrics at Term-Equivalent Age and Developmental Outcome in Extremely Low-Birth-Weight Infants. *Am J Perinatol* 2015; **32**: 1177-84.

20. Park H, Yoon H-K, Han S, et al. Brain MRI measurements at a term-equivalent age and their relationship to neurodevelopmental outcomes. *AJNR Am J Neuroradiol* 2014; **35**: 599-603.

21. Tich SN, Anderson PJ, Hunt RW, Lee KJ, Doyle LW, Inder TE. Neurodevelopmental and perinatal correlates of simple brain metrics in very preterm infants. *Arch Pediatr Adolesc Med* 2011; **165**: 216-22.

22. Kidokoro H, Neil JJ, Inder TE. New MR imaging assessment tool to define brain abnormalities in very preterm infants at term. *AJNR Am J Neuroradiol* 2013; **34**: 2208-14.

23. Brouwer MJ, van Kooij BJ, van Haastert IC, et al. Sequential cranial ultrasound and cerebellar diffusion weighted imaging contribute to the early prognosis of neurodevelopmental outcome in preterm infants. *PLoS One* 2014; **9**: e109556.

24. Murray AL, Scratch SE, Thompson DK, et al. Neonatal brain pathology predicts adverse attention and processing speed outcomes in very preterm and/or very low birth weight children. *Neuropsychology* 2014; **28**: 552-62.

25. Omizzolo C, Scratch SE, Stargatt R, et al. Neonatal brain abnormalities and memory and learning outcomes at 7 years in children born very preterm. *Memory* 2014; **22**: 605-15.

26. George JM, Boyd RN, Colditz PB, et al. PPREMO: a prospective cohort study of preterm infant brain structure and function to predict neurodevelopmental outcome. *BMC Pediatr* 2015; **15**: 123.

27. Walsh JM, Doyle LW, Anderson PJ, Lee KJ, Cheong JL. Moderate and late preterm birth: effect on brain size and maturation at term-equivalent age. *Radiology* 2014; **273**: 232-40.

28. Olsen JE, Brown NC, Eeles AL, et al. Early general movements and brain magnetic resonance imaging at term-equivalent age in infants born <30weeks' gestation. *Early Hum Dev* 2016; **101**: 63-8.

29. Martin JA, Osterman MJ, Kirmeyer SE, Gregory EC. Measuring Gestational Age in Vital Statistics Data: Transitioning to the Obstetric Estimate. *Natl Vital Stat Rep* 2015; **64**: 1-20.

30. Bayley N. Bayley Scales of Infant and Toddler Development–Third Edition. San Antonio, TX: Harcourt Assessment; 2006.

31. Burns YR, Ensbey RM, Norrie MA. The Neuro-Sensory Motor Developmental Assessment: Part 1. Development and administration of the test. *Aust J Physiother* 1989; **35**: 141-9.

32. Burns YR, Ensbey RM, Norrie MA. The Neuro Sensory Motor Developmental Assessment Part II: Predictive and concurrent validity. *Aust J Physiother* 1989; **35**: 151-57.

33. Burns Y, O'Callaghan M, McDonell B, Rogers Y. Movement and motor development in ELBW infants at 1 year is related to cognitive and motor abilities at 4 years. *Early Hum Dev* 2004; **80**: 19-29.

34. Spittle AJ, Lee KJ, Spencer-Smith M, Lorefice LE, Anderson PJ, Doyle LW. Accuracy of Two Motor Assessments during the First Year of Life in Preterm Infants for Predicting Motor Outcome at Preschool Age. *PLoS One* 2015; **10**: e0125854.

35. MacDonald J, Burns Y. Performance on the NSMDA during the first and second year of life to predict functional ability at the age of 4 in children with cerebral palsy. *Hong Kong Physiother J* 2005; **23**: 40-5.

36. Hack M, Breslau N, Aram D, Weissman B, Klein N, Borawski-Clark E. The effect of very low birth weight and social risk on neurocognitive abilities at school age. *J Dev Behav Pediatr* 1992; **13**: 412-20.

37. Roberts G, Howard K, Spittle AJ, Brown NC, Anderson PJ, Doyle LW. Rates of early intervention services in very preterm children with developmental disabilities at age 2 years. *J Paediatr Child Health* 2008; **44**: 276-80.

38. Treyvaud K, Ure A, Doyle LW, et al. Psychiatric outcomes at age seven for very preterm children: rates and predictors. *J Child Psychol Psychiatry* 2013; **54**: 772-9.

39. Solaski M, Majnemer A, Oskoui M. Contribution of socio-economic status on the prevalence of cerebral palsy: a systematic search and review. *Dev Med Child Neurol* 2014; **56**: 1043-51.

40. Ward K. Perceived needs of parents of critically ill infants in a neonatal intensive care unit (NICU). *Pediatr Nurs* 2001; **27**: 281-6.

41. Pearce R, Baardsnes J. Term MRI for small preterm babies: do parents really want to know and why has nobody asked them? *Acta Paediatr* 2012; **101**: 1013-5.

42. Janvier A, Barrington K. Trying to predict the future of ex-preterm infants: who benefits from a brain MRI at term? *Acta Paediatr* 2012; **101**: 1016-7.

43. Skiold B, Eriksson C, Eliasson AC, Aden U, Vollmer B. General movements and magnetic resonance imaging in the prediction of neuromotor outcome in children born extremely preterm. *Early Hum Dev* 2013; **89**: 467-72.

44. Bosanquet M, Copeland L, Ware R, Boyd R. A systematic review of tests to predict cerebral palsy in young children. *Dev Med Child Neurol* 2013; **55**: 418-26.

45. Setanen S, Lahti K, Lehtonen L, et al. Neurological examination combined with brain MRI or cranial US improves prediction of neurological outcome in preterm infants. *Early Hum Dev* 2014; **90**: 851-6.

4.3 Summary of Chapter 4

This paper provides validation of a scoring system for Early structural MRI at 29-35 weeks PMA for infants born <31 weeks GA. Strengths of the study include the large sample with Early MRI and 12-month motor and cognitive outcomes, which is representative of contemporaneous cohorts of preterm infants born <31 weeks GA. While this thesis focusses on motor outcomes, cognitive outcomes were included in this paper to adequately validate the scoring methodology.

This study provides a number of key contributions. It is the first validated scoring system for Early structural MRI that includes evaluation of deep GM structures and the cerebellum. It is also the first validated scoring system for Early MRI that utilises regional measurements to capture the impact of secondary brain growth impairments following early brain injury. Associations with 12-month CA motor and cognitive outcomes are presented, as well as sensitivity and specificity as measures of diagnostic accuracy for detection of infants with later adverse motor outcomes.

The present study provides detailed evaluation of the relationship between each regional brain measurement and PMA at time of MRI and proposes a scoring algorithm to correct for PMA at MRI for each measure. This is important as a method to adequately adjust for PMA at MRI in statistical analyses. The equations for correcting the regional measurements proposed here effectively standardise the PMA at MRI allowing for accurate comparison between infants. In addition, cut points of regional measures are based on mean and standard deviation data of the term reference sample, a modification to the original scale upon which this scoring system is based. A scoring proforma is provided to facilitate clinical and research utility of the tool.

The application of this scoring system was descriptive and not interpretive. Findings were scored objectively without interpretation of the aetiology or likely significance of a particular finding in regards to outcome. The reason for this was to account for situations where the aetiology was not straightforward, and differences in interpretation would result in a reduction of the reliability of the scoring system. This makes the scoring system accessible to a wider range of clinicians, and not limited to only those with very extensive knowledge of radiology. Isolated findings which are known to be incidental findings with limited sequelae, will fall into the normal/mild range in this scoring system. An example of this is ON-LINE Figure 2 which shows bilateral connatal cysts, a common incidental finding of no clinical significance. We have amended the caption for figure 2 to “Bilateral connatal cysts, classified as cerebral WM, cystic lesion, focal bilateral, score 2 (axial

T2)', to ensure that readers do not mistake this example as cystic periventricular leukomalacia, which carries a much more significant risk of adverse outcomes.

Limitations of the study include the relatively low sensitivity of the scoring system to predict motor and cognitive outcomes. The specificity of the scoring system is reasonable, indicating that a normal or mild MRI abnormality score determines which infants progress to a normal outcome, and there is a low risk of false positives. The sensitivity for Early MRI to predict cognitive outcomes presented here is still higher than that of the most widely used qualitative MRI scoring system at TEA (50% vs 41%) [6]. The fundamental reality is that brain macrostructure evaluated qualitatively for evidence of injury and/or growth impairment, incorrectly classifies a number of infants. Some infants with quite severe brain lesions, especially enlarged ventricles, go on to have reasonable motor and cognitive outcomes, while others with qualitatively 'normal' brains continue on to display poor outcomes. This is one of the reasons that advanced diffusion MRI is gaining traction in an attempt to determine if brain microstructure can address this challenge and improve diagnostic accuracy. Interestingly, sensitivity is higher at Early MRI than Term MRI, while specificity is slightly higher at Term rather than Early MRI in the present study. Despite these limitations, the potential to identify high risk infants prior to discharge from the NICU and reduce the burden to families of having to return to an advanced medical centre for neuroimaging is not to be underestimated. It would enable planning of follow-up care and implementation of targeted, early interventions to optimise outcomes for these vulnerable infants and their families. If false positives do occur, it will result in increased follow up and care. This situation is preferable to a loss of follow up that may occur from families failing to return for an MRI at TEA and thereby never being identified at higher risk of adverse outcomes.

Following validation of the scoring system for use at Early MRI (29-35 weeks PMA), the next step taken was to examine the relationships between MRI scores and concurrent clinical measures at Early and Term MRI to address Aim 3 of the thesis.

Chapter 5: Relationship between very early brain structure and neuromotor, neurological and neurobehavioral function in infants born <31 weeks gestational age

5.1 Introduction to Chapter 5

To examine the relationships between the structural MRI scores and concurrent clinical measures of motor, neurological and neurobehavioural performance, cross-sectional analysis was conducted with Early MRI and the clinical measures conducted within a week of the Early MRI. This was then repeated for the Term MRI and concurrent clinical assessments. The results are presented in the following paper.

5.2 Paper 4:

This manuscript has been submitted to the journal *Early Human Development* (journal impact factor 1.913).

George JM, Fiori S, Fripp J, Pannek K, Guzzetta A, David M, Ware RS, Rose SE, Colditz PB, Boyd RN. Relationship between very early brain structure and neuromotor, neurological and neurobehavioral function in infants born <31 weeks gestational age.

Relationship between very early brain structure and neuromotor, neurological and neurobehavioral function in infants born <31 weeks gestational age

George JM, Fiori S, Fripp J, Pannek K, Guzzetta A, David M, Ware RS, Rose SE, Colditz PB, Boyd RN

Abstract

Aim This study aimed to examine associations between structural MRI and concurrent motor, neurological and neurobehavioural measures at 30-32 weeks postmenstrual age (PMA; 'Early'), and at term equivalent age ('Term').

Method In this prospective cohort study, infants underwent Early MRI (n=119; 73 male; median 32weeks 1 day PMA) and Term MRI (n=102; 61 male; median 40 weeks 4 days PMA) at 3T. Structural images were scored generating white matter (WM), cortical gray matter, deep gray matter, cerebellar and global brain abnormality scores. Clinical measures were General Movements Assessment (GMs), Hammersmith Neonatal Neurological Examination (HNNE) and NICU Neonatal Neurobehavioral Scale (NNNS). The Premie-Neuro was administered Early and the Test of Infant Motor Performance (TIMP) and a visual assessment at Term.

Results Early MRI cerebellar scores were strongly associated with neurological components of HNNE (reflexes), NNNS (Hypertonicity), the Premie-Neuro neurological subscale (regression coefficient β=-0.06; 95% confidence interval CI=-0.09, -0.04; p<0.001) and cramped-synchronized GMs (β=1.10; 95%CI=0.57, 1.63; p<0.001). Term MRI WM and global scores were strongly associated with the TIMP (WM β=-1.02; 95%CI=-1.67, -0.36; p=0.002; global β=-1.59; 95%CI=-2.62, -0.56; p=0.001).

Interpretation Brain structure on Early and Term MRI was associated with concurrent motor, neurological and neurobehavioral function in very preterm infants.

What this paper adds

Structure-function relationships exist between MRI abnormality scores and concurrent clinical measures at both Early and Term MRI.

At Early MRI, cerebellar subscale scores have the strongest associations with clinical measures.

Early MRI cerebellar scores relate to neurological and motor rather than neurobehavioural items.

At Term MRI, the strongest associations were with motor performance on the TIMP.

WM abnormality scores are related to motor and neurological performance at Term but not at Early MRI.

Infants born very preterm are at high risk of impaired motor, cognitive, language and behavioral function which are the result of early brain injury and impaired brain development. Brain imaging such as magnetic resonance imaging (MRI) and clinical evaluation (motor, neurological or neurobehavioural function) are different techniques to identify structural and functional markers of brain injury and development. Both methods are used to predict outcomes, target interventions and counsel and support families[1-4]. Relationships between these brain structure and function methods have been demonstrated at term equivalent age (TEA) in very preterm infants[5-7]. Although MRI is now more frequently acquired earlier than TEA, there is little information yet on structure-function relationships at this earlier stage. Availability of clinical correlates for this early structural MRI would support clinicians working without access to MRI, and guide selection of clinical measures to discriminate between infants with structural brain abnormalities and those without.

At TEA, structure-function relationships have been demonstrated between qualitative structural MRI scoring systems and clinical measures of motor (General Movements Assessment, GMs), neurological (Hammersmith Neonatal Neurological Examination, HNNE) and neurobehavioural function (NICU Network Neurobehavioral Scale, NNNS)[5-8]. The MRI scoring systems utilized in these studies evaluate white matter (WM) and cortical gray matter (GM) for evidence of injury. Cerebral WM abnormalities, the predominant pattern of brain injury in very preterm infants, are associated with poorer neurological and neurobehavioral scores at TEA[5-7]. Earlier MRI studies with qualitative scoring of structural images demonstrate associations with later neurodevelopmental outcomes [9-11]; however concurrent functional correlates have not yet been demonstrated.

Scoring systems of structural MRI at TEA have been further developed to include evaluation of deep GM structures and the cerebellum, and include regional measurements to capture the effect of impaired brain growth[12]. Validated for use from 36-42 weeks postmenstrual age (PMA), the scale demonstrates associations with gestational age (GA) at birth, birthweight, a number of clinical risk factors and neonatal infection[12, 13]. No concurrent motor, neurological or neurobehavioural correlates have been published for this scoring system at TEA. This scoring system, which includes evaluation of deep GM and the cerebellum as well as incorporating regional measurements, has recently been adapted and validated for use from 29-35 weeks PMA in very preterm infants[11]. These comprehensive scoring systems of structural MRI provide new biomarkers of brain injury and development in preterm infants.

The aim of this study was to examine the structure-function relationships between structural MRI brain abnormality scores and concurrent clinical measures of neuromotor, neurological and

neurobehavioral performance at 30-32 weeks PMA ('Early' MRI) and again at 40-42 weeks PMA ('Term' MRI). A secondary aim was to evaluate which clinical measures demonstrated the strongest association with a) Early MRI and b) Term MRI.

Method

Study Design and Participants

This prospective cohort study enrolled infants born <31 weeks GA at the specialist tertiary neonatal center at the Royal Brisbane and Women's Hospital between February 2013 and February 2016. Infants were eligible if their parents/carers lived within a 200 km radius of the hospital and were English speaking. Infants with known congenital or chromosomal abnormalities likely to affect their neurodevelopmental outcome were excluded. Informed parental consent was obtained for all participants. This study is nested within a broader study, and sample size calculations are detailed in the study protocol[14]. Ethical approval was obtained from the RBWH Human Research Ethics Committee (HREC/12/QRBW/245), The University of Queensland (2012001060) and the trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12613000280707).

MRI Acquisition

Brain MRI was performed between 30-32 weeks PMA or when the infant was medically stable ('Early'), and again at 40-42 weeks PMA ('Term'). Infants were scanned utilizing an MR compatible incubator equipped with a dedicated neonatal head coil (LMT Lammers Medical Technology, Lübeck, Germany). MRI was performed during natural sleep without sedation, and with ear protection to attenuate noise. A 3T MRI Siemens Tim Trio (Erlangen, Germany) scanner was used. Coronal, axial, and sagittal T2-weighted HASTE (TR/TE 2000/90ms, flip angle 150°, field of view 200x160mm, matrix 320x256, slice thickness 4mm) were acquired as they are more resilient to motion artefacts . Axial T1 TSE (TR/TE 1490/90ms, flip angle 150°, field of view 200x160mm, matrix 256x180, slice thickness 2mm) and an axial multi-echo T2 TSE (TR/TE1/TE2/TE3 10580/27/122/189ms, flip angle 150°, field of view 144x180mm, matrix 204x256, slice thickness 2mm) were acquired.

MRI Scoring

A standardized MRI scoring system was used to score all MRIs by an independent neurologist with training in radiology (SF)[11, 12]. The scorer was blinded to birth and medical history, cranial ultrasound results and clinical assessment findings. Four subscale scores were generated; WM, cortical GM, deep GM, and the cerebellum, the total of which produced a global score[11, 12]. Cerebral WM scoring evaluates cystic degeneration, focal signal abnormalities, delayed myelination,

thinning of the corpus callosum, dilated lateral ventricles, and reduction of WM volume. Cortical GM assesses signal abnormality, delayed gyration, and dilated extracerebral CSF space. Signal abnormality and volume reduction of the deep GM and cerebellum are evaluated and scored. Both T1 and T2 images were evaluated during scoring. T1 hyperintensities and T2 hypointensities were both recorded and considered as signal abnormalities. Sagittal T2 weighted images were used to score the corpus callosum as it is clearly visualized as low signal intensity prior to myelination. Inter- and intra-rater reproducibility of the scale have been demonstrated[11, 12].

Clinical Measures

Clinical assessments were completed within a week of MRI. Tools were combined to reduce handling of the infant. At Early assessment the GMs, HNNE, NNNS and the Premie-Neuro were conducted and assessments were modified with items inappropriate for administration removed. At TEA, all assessments were completed in full (GMs, NNNS, HNNE, the Test of Infant Motor Performance TIMP, and a visual assessment). Clinical assessors were blinded to birth history and brain imaging findings.

The GMs evaluates neuromotor performance through observation of spontaneous movements and good predictive validity has been reported. Sensitivity in the preterm period and at TEA is 75-100%, with higher sensitivity for an outcome of cerebral palsy (CP) than general developmental outcomes; specificity ranges from 40-48%[15, 16]. Scoring was performed by advanced GMs raters JG and BS, with BS additionally blinded to other clinical assessment findings. Cases of non-agreement were reviewed until consensus was reached and advice sought from a third rater (blinded to all clinical and imaging information except PMA at assessment) where necessary.

The HNNE is a neurological assessment evaluating posture, tone, reflexes, spontaneous movements, orientation and behaviour[17]. All items except placing were administered at the Early assessment. When performed in the preterm period, reported sensitivity and specificity for predicting an outcome of CP are 57-86% and 45-83% respectively, increasing to 68-96% and 52-97% respectively when administered at TEA[18]. Inter-rater reliability between the clinical assessor (JG) and an observer (PC) for the HNNE total optimality score was tested with the intra-class correlation coefficient calculated to be 0.94 Early, and 0.99 at Term.

The NNNS is a neurobehavioral assessment that evaluates an infant's response to stimuli and handling, state regulation, motor performance and neurological status[19]. For administration at Early assessment, a number of items were removed which resulted in availability of summary scores in 10

of the 13 domains of the test. The NNNS at TEA has been shown to predict motor and cognitive outcomes at 18 months corrected age (CA), motor outcomes at 24 months CA and cognitive outcomes at 4.5 years[20-22]. Cerebral abnormalities correlated with poorer NNNS scores at TEA[5]. The test administrators (JG and KM) are accredited on the NNNS.The Premie-Neuro (PN) is a neurological examination designed for use from 23-37 weeks PMA in preterm infants[23]. It could be scored from the combination of the other Early assessments with the addition of only a single item. The PN consists of 3 categories; neurological, movement and responsiveness, and has scoring based on expected performance at each week of PMA[23].

At TEA, a visual assessment developed by Ricci *et al* was used to examine visual function by testing ocular motility, acuity and the ability to fix and follow[24]. Visual function demonstrates predictive validity for neurodevelopmental outcomes in preterm cohorts[25]. The TIMP was introduced as a standardized assessment of gross motor development. Construct validity enabling discrimination between infants at high and low risk of adverse motor outcomes has been demonstrated[26]. Sensitivity for prediction of school age motor outcomes has been reported at 50%, and specificity of 100%[27].

Statistical Analysis

Associations between each MRI subscale and global score and each concurrent clinical measure were evaluated using linear regression. This was performed separately for the Early and Term MRI data and the respective concurrent clinical data. Univariable analysis was performed, followed by multivariable analysis adjusting for GA at birth, sex and a measure of social risk[14, 28]. Results are presented as regression coefficients with 95% confidence intervals and the level of significance was set at 5%. There was no imputation for missing data and appropriateness of regression models was assessed using standard diagnostic tests. Analysis was performed using the Stata statistical software package, version 14 (StataCorp, College Station, TX, USA).

Results

Of 323 eligible preterm infants, 146 consented to the current study and 119 infants had Early MRI and clinical assessments completed and were included in this analysis (7 became medically unstable, 1 died, 7 cancelled due to MRI equipment failures, 7 MRI slots unavailable, 1 withdrew, 2 had an MRI-incompatible surgical clip and 2 unsuccessful MRIs due to movement artefact). Of these, 102/119 infants also had MRI and clinical data available at Term (10 failed to attend, 4 had clinical assessment but no MRI - 2 MRI's cancelled due to technical equipment difficulties, 1 declined Term MRI, 1 hospitalized remotely at Term; 3 excluded as PMA at MRI>42 weeks).

Statistical analysis of the birth and maternal characteristics of the 17 infants without a term MRI compared with the 102 with a term MRI, revealed no significant differences except for social risk. A higher social risk has been demonstrated to be associated with poorer neurodevelopmental outcomes and an increased risk of cerebral palsy[28, 29], and so all multivariable analyses included social risk as a covariate. Demographic and perinatal details of the included cohort are summarized in Table 1; MRI and clinical assessment scores are presented in Table 2.

Early MRI structure-function relationships

Results of multivariable regression analyses between Early MRI and concurrent clinical measures are presented in Table 3; results of univariable analyses are presented in Supplementary Table 1. Strongest associations were between cerebellar scores and HNNE Reflexes (β=-0.17; 95%CI=-0.30, -0.05; *p*=0.006), NNNS Hypertonicity (β=0.49; 95%CI=0.18, 0.80; p=0.002), Premie-Neuro Neurological subscale (β=-0.06; 95%CI=-0.09, -0.04; *p*<0.001) and cramped-synchronized GMs (β=1.10; 95%CI=0.57, 1.63; *p*<0.001). Cortical GM scores were associated with the HNNE subscales of Posture and Tone (Regression coefficient β=-0.11; 95% confidence interval CI=-0.19, -0.03; *p*=0.008) and Tone Patterns and the NNNS subscales of Regulation and Hypotonicity. Deep GM was associated with Stress on the NNNS.

Term MRI structure-function relationships

Results of multivariable regression analyses between Term MRI and concurrent clinical measures are presented in Table 4; results of univariable analyses are presented in Supplementary Table 2. White matter was associated with the TIMP (β=-1.04; 95%CI=-1.71, -0.38; *p*=0.002), HNNE Abnormal Signs and NNNS Hypertonicity. Cortical GM was associated with HNNE Tone patterns and Orientation and Behavior. Deep GM was associated with the TIMP and HNNE Orientation and Behavior. Cerebellar scores were associated with HNNE Posture and Tone and NNNS Hyper- and Hypotonicity. Global scores were associated with the TIMP (β=-1.62; 95%CI=-2.66, -0.58; *p*=0.003), HNNE abnormal signs and NNNS Hypertonicity. No associations were found between any MRI subscale scores and the GMs or visual scores.

Discussion

This study is, to our knowledge, the first to present structure-function relationships between Early structural MRI and concurrent clinical measures of motor, neurological and neurobehavioral function in infants born very preterm. It is also the first study with clinical correlates of a structural Early MRI scoring system that includes evaluation of deep GM and the cerebellum. Of the Early MRI subscale scores, the cerebellar scores were most strongly associated with clinical measures.

Neurological and motor items were the predominant functional correlates found for the Early cerebellar scores. These findings contribute to a growing body of evidence of the vital role of the cerebellum in early neurodevelopment[30]. The fact that cerebellar abnormality is associated with neurological test items demonstrates the likely important role of the cerebellum in mediating neurological function during this critical period of development. To our knowledge, these are the first direct functional correlates of Early cerebellar structural abnormality. It is pertinent to interpret these findings with caution; further follow up is necessary to determine if the structure-function relationships presented here are maintained as infants get older, and whether they represent clinically important differences related to longer term outcomes.

Cerebellar scores in the scoring system employed here consist of evaluation of signal abnormality and volume reduction[11, 12]. Between Early and Term MRI a small proportion of signal abnormalities resolved (n=3), while volume reduction remained stable at 17%. The rate of cerebellar development surpasses most other structures between 24-40 weeks PMA and it is of interest that cerebellar volume reduction was already present at Early MRI in 17% of our cohort[31].

The lack of associations between Early WM scores and clinical measures is of particular interest. In contrast, the majority of Term MRI studies have found WM abnormalities to correlate with concurrent clinical presentations and predict later neurodevelopmental outcomes[5, 6, 32]. The present study confirms this with significant associations found between Term WM scores and motor function on the TIMP, as well as neurological features on the HNNE (Abnormal Signs) and NNNS (Hypertonicity). As research is moving from qualitative evaluation of structural MRI to more advanced diffusion and volumetric imaging, the focus has remained on WM injury, development and maturation. The data presented here supports inclusion of the cerebellum and deep gray matter in Early MRI studies[30].

Cortical GM scores at Early MRI demonstrate associations with the neurological elements of posture, tone and hypotonicity and the neurobehavioural feature of regulation. It must be noted that this subscale has the lowest reliability of the MRI subscales and so these relationships should be interpreted with caution[11].

At Term MRI, structure-function relationships were found for all MRI subscale scores. Of the subscales, WM and global scores demonstrated the strongest associations with clinical measures, predominantly with the TIMP which is a motor assessment tool. Term WM abnormality has been demonstrated to be significantly associated with motor performance on the TIMP at 10-15 weeks

CA[33]. We have demonstrated that these associations are present concurrently at TEA. Term MRI cerebellar scores were associated with neurological test items (HNNE Posture and Tone; NNNS Hyper- and Hypotonicity).

The secondary aim of this study was to evaluate which of the clinical measures demonstrated the strongest relationships with Early and Term MRI scores. At Early MRI, no single clinical tool showed substantial associations with MRI scores, although the Premie-Neuro neurological subscale and cramped-synchronized GMs demonstrated strong associations with cerebellar scores ($p<0.001$). At Term, the TIMP demonstrated the strongest associations of the tools with MRI. Follow up is required to determine if use of these clinical measures afford meaningful contributions to clinical practice.

A large number of statistical comparisons were undertaken in this study. All were based on robust hypotheses that clinical presentations of motor, neurological or neurobehavioural performance would be correlated with structural brain abnormalities measured by this comprehensive structural MRI scoring system. Analyses are exploratory in an area where very little published data exist, and so no correction for multiple comparisons was performed to ensure that all significant associations were identified. While it is pertinent to remain cautious about these findings, biological plausibility is suggested by the consistency with which similar items from different clinical measures were found to demonstrate significant associations with the MRI scores, for example neurological items from different clinical tests with both Early and Term MRI cerebellar scores. We have been careful to emphasize only the strongest associations throughout the results and discussion of this paper ($p<0.01$). Ultimately, replication of the study is required to determine if these findings are reproducible in other cohorts of preterm infants.

Strengths of the current study include the large sample of Early MRI data coupled with concurrent clinical data of infants born very preterm, in a contemporaneous study cohort with blinded clinical and MRI assessment. Limitations of the study include the relatively wide age range at Early MRI. The study protocol set the window for MRI at 30-32 weeks PMA, with sicker and more fragile infants undergoing MRI once they became medically stable and up to a maximum PMA of 36 weeks. This ensured that sicker infants were included in the sample, which was necessary for our results to be generalizable to other populations of very preterm infants. The MRI scoring system has been rigorously designed to account for brain changes in size and volume that are the result of variable PMA at MRI, thereby minimizing potential scoring bias due to PMA at MRI[11]. Another potential limitation is our use of an established scoring system for structural images rather than

more complex volumetric or diffusion based systems. This is a clinically accessible MRI scoring system to examine associations that may be present with clinical bedside measures and is an important first step in understanding Early MRI data. It is less resource-intensive and more readily clinically available than advanced MRI measures. It also enables classification of all MRI's in a cohort whereas advanced diffusion imaging frequently excludes participant MRI's due to artefact. Further evidence of clinical utility is required of both the early MRI scoring system employed in this study, and the concurrent clinical measures, to determine the extent to which these findings may contribute to clinical patient care. Our MRI acquisition techniques used a slice thickness of 4mm which may be a potential limitation, as some subtle abnormalities may have been missed. As this is a qualitative scoring system, we anticipate that the majority of injuries were detected and scored appropriately. Gradient echo techniques have been shown to be superior to conventional techniques in detection of cerebellar abnormalities. The T1 gradient echo sequence is very disruptive to the infant, often waking them up, although we did try to acquire the sequence at the end of the scan. The scorer (SF), in each case, selected the most appropriate image (whether T1 or T2) that best showed the underlying pathology for scoring. We recognize that an important future step is to report the longer term outcomes of this cohort. Cognitive and motor outcomes to 12 months CA for a subset of the present cohort are available and longer term follow up is underway[11]. Future work will investigate the relationship between volumetric, cortical thickness and cortical folding measures and the clinical assessments.

Conclusion

Structure-function relationships exist between structural MRI and concurrent clinical measures of motor, neurological and neurobehavioural function both Early and at Term in infants born preterm. At Early MRI, cerebellar subscale scores have the strongest associations with clinical measures. Early MRI cerebellar scores relate to neurological and motor rather than neurobehavioural items. At Term MRI, the strongest associations were with motor performance on the TIMP. White matter abnormality scores are related to motor and neurological performance at Term but not at Early MRI. These findings are an important contribution to the understanding of very early brain structure-function relationships in preterm infants.

Acknowledgements

We would like to acknowledge the families of preterm infants who participated in this study, the medical and nursing staff of the neonatal unit and radiology and administrative staff in the department of medical imaging. We also acknowledge the contribution of Dr Melissa Lai who undertook clinical study management and escorted infants to MRI, research nurses Donna Hovey,

Kellie McGrory and Kylie Smart who conducted recruitment, study management and infant MRI, Kym Morris who performed clinical assessment and Bernadette Shannon and Christine Finn who independently scored GMs.

Table 1: Characteristics of the study sample included in paper 4

Birth and Maternal Data	**Sample with Early MRI n=119** **n (%), Median [25th-75th centiles] or Mean (SD), range**	**Sample with additional Term MRI n=102**
Gestational age at birth (weeks-w, days-d)	28w3d [26w6d-29w3d], range 23w1d – 30w6d	28w5d [26w5d-29w4d], range 23w6d – 30w6d
Birth weight (g)	1093 (321), range 494 – 1886	1079 (329), range 494 – 1886
Birth head circumference (cm)	25.77 (2.36), n=114	25.68 (2.43), n=98
Males	73 (61%)	61 (60%)
Multiple births	36 (30%)	29 (28%)
Premature rupture of membranes	27 (23%)	21 (21%)
Caesarian section	84 (71%)	75 (74%)
Chorioamnionitis	18 (15%)	16 (16%)
Antenatal steroids	83 (70%)	72 (71%)
Magnesium sulphate	63 (64%), n=98	56 (66%), n=85
Higher social risk	58 (49%), n=117	46 (45%)
Acquired medical factors	**From birth to Early MRI**	**From birth to Term MRI**
Patent ductus arteriosus	59 (50%)	54 (53%)
Any intraventricular hemorrhage	30 (25%)	26 (25%)
Intraventricular hemorrhage grade III or IV	8 (7%)	8 (8%)
Periventricular leukomalacia	4 (3%)	4 (4%)
Hydrocephalus	4*(3%)	4 (4%)
Seizures treated with anticonvulsant therapy	1 (1%)	1 (1%)
NEC diagnosed or suspected	5 (4%)	4 (4%)
Confirmed sepsis	5 (4%)	4 (4%)
Total parenteral nutrition (days)	11 [7–14], range 0 – 36	11 [9-15], range 0-36
Postnatal corticosteroids	20 (17%)	19 (19%)
Ventilation (days)	2 [0–10], range 0 – 50	2 [0–15], range 0 – 50
CPAP (days)	14 [7–25], range 0 – 47	26 [7–47], range 0 – 81
Oxygen therapy (hours)	37 [2–210], Range 0 – 1515, n=105	63 [3–543], range 0 – 3912, n=92
Bronchopulmonary dysplasia[#]		32 (31%)
PMA at MRI (weeks-w, days-d)	32w1d (1w3d), range 29w3d – 35w2d	40w4d (1w), range 38w3d – 42w5d
Weight at MRI (g)	1500 (340), range 858-2715	3019 (510), range 1900 – 4300
PMA at clinical assessment (weeks-w, days-d)	32w3d (1w3d), range 29w4d – 36w3d	40w6d (1w1d), range 38w4d – 44w1d

Key: PMA postmenstrual age; NEC necrotizing enterocolitis; CPAP continuous positive airway pressure; [#]defined as oxygen requirement at 36 weeks; *All 4 infants with hydrocephalus also had IVH grade III/IV.

Table 2: Summary of MRI and clinical scores for the sample included in paper 4

		Preterm sample with Early MRI n=119	**Preterm sample with additional Term MRI N=102**
MRI Scores median [25th-75th centiles]			
WM ↓		3 [2-5]	2 [1-3]
Cortical GM ↓		0 [0-1]	0 [0-1]
Deep GM ↓		0 [0-1]	0 [0-1]
Cerebellum ↓		0 [0-0]	0 [0-1]
Global ↓		4 [3-7]	3 [1-5]
GMs n (%)			n=97
Normal		39 (33%)	31 (32%)
Poor Repertoire		72 (61%)	57 (59%)
Cramped Synchronized		8 (7%)	9 (9%)
HNNE n, mean (SD)			
Posture & Tone ↑	n=111	3.80 (1.90)	6.85 (1.67)
Tone Patterns ↑	n=111	3.91 (0.78)	3.65 (0.84)
Reflexes ↑	n=113	2.43 (0.99)	4.18 (1.13)
Spontaneous movements ↑	n=110	1.04 (0.84)	2.29 (0.79)
Abnormal signs ↑	n=119	2.03 (0.60)	2.55 (0.53)
Orientation & Behavior ↑	n=118	2.96 (1.49)	5.19 (1.26)
HNNE total score↑	n=109	16.17 (3.73)	24.71 (3.82)
NNNS mean (SD)			n=100
Quality of movement ↑		3.43 (0.61)	4.38 (0.57)
Regulation ↑	n=118	4.14 (0.61)	4.95 (0.63)
Nonoptimal reflexes ↓		6.96 (1.50)	6.61 (2.80)
Stress/Abstinence↓		0.22 (0.07)	0.18 (0.07)
Arousal ↑↓		3.11 (0.57)	4.26 (0.56)
Hypertonicity ↓		0.12 (0.39)	0.19 (0.60)
Hypotonicity ↓		1.54 (1.15)	0.50 (0.72)
Asymmetric reflexes ↓		0.92 (0.95)	0.75 (0.98)
Excitability ↓		2.94 (1.71)	3.67 (1.84)
Lethargy ↓		8.48 (1.96)	5 (2.19)
Premie-Neuro n, mean (SD)			
Factor 1 Neurological ↑		31.63 (4.28)	
Factor 2 Movement ↑	n=118	34.20 (4.57)	
Factor 3 Responsiveness ↑	n=111	31.33 (3.54)	
Total score ↑	n=111	97.42 (7.79)	
TIMP mean (SD)			
z-score ↑			-0.60 (0.66)
Visual Score; n, mean (SD)			
Total score ↑			15.95 (6.13)

Key: WM white matter; GM gray matter; GMs General Movements Assessment; HNNE Hammersmith Neonatal Neurological Examination; NNNS NICU Neonatal Neurobehavioral Scale; TIMP Test of Infant Motor Performance; SD standard deviation; IQR Interquartile range; ↑ higher scores better; ↓ lower scores better.

Table 3: Multivariable regression results of relationships between Early MRI scores and concurrent clinical data (model covariates: GA at birth, sex, social risk). N=118 as 1 participant had no social risk data available.

	Early MRI Scores														
	WM			Cortical GM			Deep GM			Cerebellum			Global		
GMs (n=118)	ß	95%CI	*p*	ß	95%CI	*p*	ß	95%CI	*p*	ß	95%CI	*p*	ß	95%CI	*p*
Normal	ref			ref			ref			ref			ref		
Poor Repertoire	-0.17	-0.93, 0.58	0.65	0.04	-0.28, 0.36	0.82	0.12	-0.31, 0.56	0.58	0.12	-0.14, 0.38	0.35	0.11	-1.13, 1.35	0.86
Cramped Synchronized	0.37	-1.17, 1.92	0.63	-0.10	-0.76, 0.55	0.75	0.07	-0.81, 0.96	0.87	1.10	0.57, 1.63	**<0.001**	1.45	-1.09, 3.98	0.26
HNNE															
Posture & Tone n=110	0.08	-0.12, 0.28	0.44	-0.11	-0.19, -0.03	**<0.01**	0.03	-0.08, 0.14	0.54	-0.01	-0.08, 0.06	0.76	-0.01	-0.34, 0.32	0.95
Tone Patterns n=110	0.09	-0.37, 0.54	0.71	0.21	0.02, 0.40	**0.03**	0.10	-0.16, 0.35	0.46	0.15	-0.01, 0.30	0.06	0.53	-0.20, 1.27	0.15
Reflexes n=112	-0.08	-0.43, 0.27	0.64	-0.05	-0.20, 0.09	0.47	-0.06	-0.24, 0.12	0.49	-0.17	-0.30, -0.05	**<0.01**	-0.37	-0.91, 0.17	0.17
Spontaneous movements n=109	-0.27	-0.70, 0.17	0.23	-0.05	-0.23, 0.14	0.62	0.02	-0.19, 0.24	0.84	-0.05	-0.21, 0.11	0.51	-0.34	-1.01, 0.33	0.32
Abnormal signs	0.03	-0.53, 0.59	0.92	0.04	-0.20, 0.28	0.72	0.21	-0.11, 0.52	0.20	-0.03	-0.24, 0.17	0.77	0.25	-0.67, 1.17	0.60
Orientation & Behavior n=117	0.07	-0.15, 0.30	0.51	0	-0.09, 0.10	0.99	0.10	-0.03, 0.22	0.13	0.01	-0.07, 0.09	0.81	0.18	-0.19, 0.55	0.33
HNNE total n=108	0.01	-0.09, 0.10	0.91	-0.03	-0.07, 0.02	0.21	0.01	-0.04, 0.06	0.73	-0.02	-0.05, 0.01	0.29	-0.03	-0.18, 0.12	0.70
NNNS															
Quality of movement	-0.16	-0.72, 0.40	0.57	0.07	-0.17, 0.30	0.59	0.18	-0.14, 0.50	0.27	-0.08	-0.28, 0.13	0.46	0.01	-0.92, 0.93	0.99
Regulation n=117	-0.31	-0.86, 0.25	0.28	-0.29	-0.52, -0.06	**0.01**	-0.03	-0.35, 0.30	0.85	-0.16	-0.36, 0.05	0.13	-0.78	-1.69, 0.12	0.09
Non-optimal reflexes	0.01	-0.21, 0.24	0.90	0.06	-0.04, 0.16	0.21	-0.04	-0.17, 0.09	0.57	0.06	-0.02, 0.14	0.13	0.10	-0.27, 0.47	0.60
Stress	-1.82	-6.77, 3.14	0.47	1.02	-1.09, 3.12	0.34	-3.11	-5.89,-0.32	**0.03**	1.73	-0.07, 3.53	0.06	-2.18	-10.36, 5.99	0.60
Arousal	0.00	-0.59, 0.59	0.99	0.08	-0.17, 0.33	0.54	-0.07	-0.41, 0.27	0.67	-0.16	-0.37, 0.06	0.15	-0.15	-1.12, 0.83	0.76
Hypertonicity	0.26	-0.62, 1.14	0.56	-0.06	-0.43, 0.32	0.76	0.15	-0.36, 0.65	0.56	0.49	0.18, 0.80	**<0.01**	0.84	-0.60, 2.28	0.25
Hypotonicity	-0.08	-0.37, 0.22	0.60	0.13	0.01, 0.25	**0.04**	-0.06	-0.23, 0.11	0.49	0.01	-0.10, 0.12	0.84	0.00	-0.48, 0.49	0.99
Asymmetric reflexes	0.05	-0.30, 0.41	0.76	0.05	-0.10, 0.20	0.53	0.18	-0.02, 0.38	0.08	-0.03	-0.16, 0.10	0.64	0.25	-0.33, 0.83	0.40
Excitability	-0.04	-0.24, 0.15	0.68	0.02	-0.06, 0.11	0.60	-0.06	-0.17, 0.05	0.27	0.01	-0.06, 0.08	0.74	-0.07	-0.39, 0.25	0.67
Lethargy	-0.02	-0.19, 0.16	0.85	0.01	-0.06, 0.09	0.77	-0.03	-0.13, 0.07	0.55	-0.03	-0.09, 0.04	0.40	-0.07	-0.36, 0.22	0.65
Premie-Neuro															
Neurological	-0.04	-0.12, 0.04	0.36	-0.01	-0.05, 0.02	0.42	-0.01	-0.06, 0.04	0.65	-0.06	-0.09, -0.04	**<0.001**	-0.13	-0.265, 0.01	0.06
Movement n=117	0.04	-0.04, 0.11	0.31	0.01	-0.02, 0.04	0.61	0.03	-0.02, 0.07	0.21	0.01	-0.02, 0.03	0.63	0.08	-0.04, 0.20	0.20
Responsiveness n=109	-0.04	-0.14, 0.06	0.46	0.01	-0.03, 0.06	0.49	0.01	-0.05, 0.07	0.72	-0.01	-0.04, 0.03	0.63	-0.02	-0.19, 0.15	0.80
Total n=109	-0.01	-0.05, 0.04	0.81	0	-0.02, 0.02,	0.95	0.01	-0.01, 0.04	0.40	-0.01	-0.03, 0	0.06	-0.01	-0.08, 0.06	0.80

Key: Early MRI, 30-32 weeks postmenstrual age (range 29-35 weeks); WM, white matter; GM, gray matter; GMs, General Movements Assessment; HNNE, Hammersmith Neonatal Neurological Examination; NNNS, NICU Neonatal Neurobehavioral Scale; CI, confidence interval; ref, reference level.

Table 4: Multivariable regression results of relationships between Term MRI scores and concurrent clinical data (model covariates: GA at birth, sex, social risk)

	Term MRI Scores														
	WM			Cortical GM			Deep GM			Cerebellum			Global		
GMs (n=97)	**ß**	**95%CI**	***p***	**ß**	**95%CI**	***p***	**ß**	**95%CI**	***p***	**ß**	**95%CI**	***p***	**ß**	**95%CI**	***p***
Normal	ref			ref			ref			ref			ref		
Poor Repertoire	-0.39	-1.45, 0.67	0.47	-0.18	-0.57, 0.21	0.37	-0.09	-0.54, 0.37	0.71	0.10	-0.24, 0.44	0.57	-0.56	-2.23, 1.12	0.51
Cramped Synchronized	-0.76	-2.56, 1.04	0.41	0.15	-0.52, 0.82	0.66	-0.32	-1.09, 0.46	0.42	-0.02	-0.60, 0.57	0.95	-0.95	-3.79, 1.90	0.51
HNNE															
Posture & Tone	-0.01	-0.28, 0.26	0.93	-0.01	-0.11, 0.09	0.79	-0.09	-0.20, 0.03	0.14	-0.11	-0.19, -0.02	**0.02**	-0.22	-0.64, 0.21	0.31
Tone Patterns	0.26	-0.27, 0.79	0.33	-0.20	-0.39, -0.01	**0.04**	0.10	-0.12, 0.33	0.37	-0.05	-0.22, 0.12	0.53	0.11	-0.72, 0.94	0.79
Reflexes	0.03	-0.37, 0.43	0.88	-0.05	-0.20, 0.09	0.48	0.05	-0.13, 0.22	0.59	-0.01	-0.14, 0.12	0.86	0.01	-0.61, 0.64	0.97
Spontaneous movements	-0.09	-0.72, 0.53	0.77	-0.13	-0.36, 0.10	0.26	-0.17	-0.44, 0.09	0.20	-0.06	-0.26, 0.14	0.58	-0.45	-1.43, 0.52	0.36
Abnormal signs	-0.96	-1.80, -0.11	**0.03**	-0.13	-0.45, 0.19	0.43	-0.28	-0.65, 0.09	0.14	-0.19	-0.47, 0.09	0.18	-1.55	-2.87, -0.23	**0.02**
Orientation & Behavior	-0.18	-0.55, 0.19	0.33	-0.14	-0.27, -0.01	**0.04**	-0.18	-0.34, -0.03	**0.02**	-0.02	-0.14, 0.10	0.73	-0.52	-1.10, 0.05	0.07
HNNE total	-0.03	-0.15, 0.09	0.64	-0.04	-0.09, 0	0.07	-0.04	-0.09, 0.01	0.13	-0.03	-0.07, 0.01	0.09	-0.14	-0.34, 0.05	0.14
NNNS n=100															
Quality of movement	-0.19	-0.99, 0.62	0.65	-0.09	-0.39, 0.21	0.54	-0.19	-0.53, 0.16	0.28	-0.07	-0.33, 0.19	0.58	-0.54	-1.80, 0.72	0.40
Regulation	-0.33	-1.07, 0.40	0.37	-0.21	-0.48, 0.06	0.12	-0.22	-0.54, 0.09	0.17	-0.07	-0.30, 0.17	0.59	-0.83	-1.98, 0.31	0.15
Nonoptimal reflexes	0	-0.17, 0.16	0.98	0.03	-0.04, 0.09	0.39	0	-0.07, 0.08	0.91	0.05	0, 0.10	0.07	0.08	-0.18, 0.34	0.56
Stress	-3.16	-9.25, 2.94	0.31	0.13	-2.14, 2.40	0.91	0.31	-2.34, 2.95	0.82	0.36	-1.62, 2.33	0.72	-2.37	-11.97, 7.24	0.63
Arousal	0.43	-0.43, 1.30	0.32	0.21	-0.11, 0.53	0.20	-0.02	-0.40, 0.35	0.90	-0.10	-0.38, 0.18	0.48	0.52	-0.84, 1.87	0.45
Hypertonicity	0.85	0.08, 1.63	**0.03**	0.16	-0.14, 0.45	0.30	0.22	-0.12, 0.56	0.21	0.26	0.01, 0.52	**0.04**	1.50	0.28, 2.70	**0.02**
Hypotonicity	0.11	-0.51, 0.74	0.71	0.19	-0.04, 0.42	0.10	0.23	-0.04, 0.50	0.09	0.21	0.01, 0.40	**0.04**	0.74	-0.23, 1.72	0.13
Asym. reflexes	-0.13	-0.60, 0.33	0.57	-0.09	-0.26, 0.08	0.31	0.10	-0.10, 0.31	0.31	0	-0.15, 0.15	1	-0.12	-0.86, 0.62	0.75
Excitability	0.11	-0.15, -0.36	0.42	0.08	-0.02, 0.17	0.12	0.02	-0.09, 0.13	0.69	-0.01	-0.10, 0.07	0.76	0.19	-0.21, 0.60	0.35
Lethargy	0.14	-0.07, 0.35	0.20	0	-0.08, 0.08	0.94	0.03	-0.06, 0.12	0.49	-0.01	-0.08, 0.06	0.84	0.16	-0.17, 0.49	0.34
TIMP															
z-score	-1.04	-1.71, -0.38	**<0.01**	-0.04	-0.30, 0.21	0.74	-0.34	-0.63, -0.05	**0.02**	-0.20	-0.42, 0.02	0.08	-1.62	-2.66, -0.58	**<0.01**
Visual n=100															
Total	0.01	-0.07, 0.08	0.85	0.01	-0.02, 0.03	0.71	-0.02	-0.05, 0.01	0.28	0.02	-0.01, 0.04	0.14	0.01	-0.11, 0.13	0.83

Key: Term MRI, 40-42 weeks postmenstrual age (range 38-42 weeks); WM, white matter; GM, gray matter; GMs, General Movements Assessment; HNNE, Hammersmith Neonatal Neurological Examination; NNNS, NICU Neonatal Neurobehavioral Scale; TIMP, Test of Infant Motor Performance; CI, confidence interval; ref, reference level; asym asymmetric.

Supplementary Tables

Supplementary Table 1: Univariable regression results of the relationships between Early MRI scores and concurrent clinical data.

	Early MRI Scores														
	WM			Cortical GM			Deep GM			Cerebellum			Global		
GMs (n=118)	**ß**	**95%CI**	***p***	**ß**	**95%CI**	***p***	**ß**	**95%CI**	***p***	**ß**	**95%CI**	***p***	**ß**	**95%CI**	***p***
Normal	ref			ref			ref			ref			ref		
Poor Repertoire	-0.07	-0.80, 0.66	0.85	0.08	-0.23, 0.39	0.62	0.17	-0.25, 0.59	0.42	0.11	-0.13, 0.36	0.36	0.29	-0.92, 1.50	0.64
Cramped	0.93	-0.50, 2.36	0.20	0.06	-0.54, 0.67	0.83	0.34	-0.48, 1.15	0.42	1.07	0.60, 1.54	**<0.001**	2.40	0.04, 4.76	**0.05**
Synchronized															*****
HNNE															
Posture & Tone n=111	-0.04	-0.23, 0.15	0.71	-0.12	-0.20, -0.05	**<0.01**	-0.01	-0.12, 0.09	0.80	-0.03	-0.09, 0.03	0.38	-0.20	-0.51, 0.11	0.21
Tone Patterns n=111	0.12	-0.34, 0.58	0.59	0.22	0.04, 0.41	**0.02**	0.11	-0.15, 0.37	0.40	0.14	-0.02, 0.29	0.08	0.59	-0.17, 1.35	0.13
Reflexes n=113	-0.16	-0.51, 0.20	0.37	-0.07	-0.22, 0.07	0.33	-0.08	-0.25, 0.10	0.40	-0.18	-0.30, -0.06	**<0.01**	-0.49	-1.04, 0.06	0.08
Spontaneous movements n=110	-0.39	-0.80, 0.03	0.07	-0.08	-0.25, 0.10	0.39	-0.09	-0.30, 0.12	0.40	-0.05	-0.20, 0.10	0.52	-0.60	-1.26, 0.06	0.07
Abnormal signs	0.09	-0.48, 0.66	0.75	0.07	-0.17, 0.31	0.55	0.22	-0.10, 0.55	0.17	-0.03	-0.24, 0.17	0.74	0.36	-0.59, 1.30	0.46
Orientation & Behavior n=118	0.09	-0.14, 0.31	0.46	0	-0.09, 0.10	0.92	0.11	-0.02, 0.23	0.11	0.01	-0.07, 0.09	0.82	0.20	-0.18, 0.59	0.29
HNNE total n=109	-0.03	-0.12, 0.07	0.55	-0.03	-0.07, 0.01	0.10	-0.01	-0.05, 0.04	0.83	-0.02	-0.05, 0.01	0.15	-0.09	-0.24, 0.06	0.24
NNNS															
Quality of movement	-0.07	-0.63, 0.50	0.81	0.09	-0.14, 0.33	0.44	0.17	-0.15, 0.49	0.29	-0.05	-0.25, 0.15	0.61	0.15	-0.79, 1.08	0.76
Regulation n=118	-0.37	-0.94, 0.19	0.19	-0.31	-0.54, -0.08	**<0.01**	-0.05	-0.37, 0.27	0.75	-0.18	-0.38, 0.02	0.08	-0.91	-1.84, 0.01	0.05
Nonoptimal reflexes	0.05	-0.18, 0.27	0.69	0.07	-0.03, 0.16	0.17	-0.03	-0.15, 0.10	0.70	0.06	-0.02, 0.14	0.15	0.15	-0.23, 0.52	0.45
Stress	-1.48	-6.53, 3.57	0.56	1.19	-0.91, 3.30	0.26	-2.90	-5.73, -0.07	**0.04**	1.68	-0.09, 3.45	0.06	-1.51	-9.92, 6.91	0.72
Arousal	0	-0.61, 0.60	0.99	0.08	-0.17, 0.34	0.51	-0.08	-0.43, 0.26	0.63	-0.15	-0.36, 0.06	0.17	-0.15	-1.15, 0.85	0.77
Hypertonicity	0.12	-0.74, 0.99	0.78	-0.10	-0.46, 0.26	0.59	0.15	-0.34, 0.64	0.55	0.46	0.17, 0.76	**<0.01**	0.63	-0.80, 2.07	0.38
Hypotonicity	-0.05	-0.35, 0.25	0.74	0.13	0.01, 0.25	**0.04**	-0.05	-0.22, 0.12	0.57	0.01	-0.10, 0.12	0.85	0.04	-0.46, 0.53	0.88
Asymmetric reflexes	0.04	-0.32, 0.40	0.82	0.05	-0.10, 0.20	0.55	0.16	-0.05, 0.36	0.13	-0.04	-0.16, 0.09	0.58	0.21	-0.39, 0.80	0.49
Excitability	-0.04	-0.24, 0.16	0.66	0.02	-0.06, 0.10	0.61	-0.06	-0.17, 0.06	0.33	0.01	-0.06, 0.08	0.73	-0.07	-0.40, 0.27	0.69
Lethargy	-0.06	-0.23, 0.12	0.53	-0.01	-0.08, 0.06	0.77	-0.04	-0.14, 0.06	0.46	-0.03	-0.09, 0.03	0.35	-0.13	-0.42, 0.16	0.37
Premie-Neuro															
Neurological	-0.07	-0.15, 0.01	0.09	-0.02	-0.06, 0.01	0.15	-0.02	-0.07, 0.02	0.37	-0.06	-0.09, -0.04	**<0.001**	-0.18	-0.31, 0.05	**0.01**
Movement n=118	0.04	-0.04, 0.11	0.36	0.01	-0.02, 0.04	0.60	0.02	-0.02, 0.07	0.28	0.01	-0.02, 0.03	0.66	0.07	-0.05, 0.20	0.25
Responsiveness n=111	0	-0.10, 0.10	0.98	0.02	-0.02, 0.07	0.27	0.02	-0.03, 0.08	0.40	0	-0.04, 0.03	0.89	0.04	-0.13, 0.21	0.61
Total n=111	-0.01	-0.06, 0.04	0.66	0	-0.02, 0.02	0.84	0.01	-0.02, 0.03	0.54	-0.02	-0.03, 0	**0.04**	-0.02	-0.10, 0.06	0.61

Key: Early MRI, 30-32 weeks postmenstrual age (range 29-35 weeks); WM, white matter; GM, gray matter; GMs, General Movements Assessment; HNNE, Hammersmith Neonatal Neurological Examination; NNNS, NICU Neonatal Neurobehavioral Scale; CI, confidence interval; ref, reference level; *p=0.047

Supplementary Table 2: Univariable regression results of the relationships between Term MRI scores and concurrent clinical data.

	Term MRI Scores														
	WM			Cortical GM			Deep GM			Cerebellum			Global		
GMs (n=97)	**ß**	**95%CI**	***p***	**ß**	**95%CI**	***p***	**ß**	**95%CI**	***p***	**ß**	**95%CI**	***p***	**ß**	**95%CI**	***p***
Normal	ref			ref			ref			ref			ref		
Poor Repertoire	0.10	-0.96, 1.16	0.85	-0.12	-0.50, 0.25	0.52	0.09	-0.37, 0.55	0.69	0.20	-0.13, 0.53	0.22	0.27	-1.44, 1.97	0.76
Cramped Synchronized	0.20	-1.59, 2.00	0.82	0.31	-0.33, 0.95	0.34	0.02	-0.76, 0.81	0.96	0.15	-0.40, 0.71	0.58	0.69	-2.20, 3.58	0.64
HNNE															
Posture & Tone	-0.10	-0.37, 0.18	0.48	-0.04	-0.14, 0.06	0.47	-0.11	-0.23, 0.01	0.07	-0.11	-0.20, -0.03	**<0.01**	-0.36	-0.80, 0.08	0.11
Tone Patterns	0.22	-0.33, 0.77	0.43	-0.22	-0.41, -0.03	**0.03**	0.07	-0.18, 0.31	0.59	-0.05	-0.22, 0.12	0.57	0.02	-0.86, 0.90	0.97
Reflexes	-0.12	-0.52, 0.29	0.57	-0.07	-0.22, 0.08	0.34	-0.01	-0.19, 0.17	0.89	-0.04	-0.17, 0.09	0.53	-0.24	-0.89, 0.41	0.47
Spontaneous movements	-0.52	-1.10, 0.06	0.08	-0.20	-0.40, 0.01	0.06	-0.33	-0.57, -0.08	**0.01**	-0.12	-0.30, 0.06	0.17	-1.17	-2.07, -0.26	**0.01**
Abnormal signs	-0.78	-1.64, 0.05	0.07	-0.05	-0.36, 0.27	0.77	-0.14	-0.52, 0.25	0.49	-0.19	-0.46, 0.08	0.17	-1.14	-2.54, 0.25	0.11
Orientation & Behavior	-0.34	-0.70, 0.02	0.06	-0.17	-0.30, -0.04	**0.01**	-0.23	-0.39, -0.08	**<0.01**	-0.05	-0.16, 0.07	0.40	-0.79	-1.36, -0.22	**<0.01**
HNNE total	-0.09	-0.21, 0.03	0.13	-0.05	-0.09, -0.01	**0.02**	-0.06	-0.11, -0.01	**0.02**	-0.04	-0.08, -0.01	**0.03**	-0.25	-0.44, -0.06	**0.01**
NNNS n=100															
Quality of movement	-0.42	-1.24, 0.41	0.32	-0.12	-0.41, 0.18	0.43	-0.25	-0.60, 0.11	0.18	-0.12	-0.37, 0.13	0.35	-0.90	-2.21, 0.41	0.18
Regulation	-0.51	-1.25, 0.24	0.18	-0.21	-0.47, 0.06	0.13	-0.30	-0.62, 0.02	0.06	-0.10	-0.33, 0.13	0.40	-1.11	-2.29, 0.06	0.06
Nonoptimal reflexes	0.05	-0.12, 0.22	0.57	0.03	-0.03, 0.09	0.33	0.01	-0.06, 0.09	0.72	0.06	0.01, 0.11	**0.03**	0.15	-0.12, 0.42	0.27
Stress	-1.44	-7.75, 4.87	0.65	0.44	-1.83, 2.71	0.70	0.77	-1.99, 3.53	0.58	0.65	-1.29, 2.59	0.51	0.42	-9.69, 10.53	0.94
Arousal	0.22	-0.62, 1.07	0.60	0.14	-0.17, 0.44	0.37	-0.02	-0.39, 0.36	0.93	-0.12	-0.38, 0.14	0.35	0.22	-1.14, 1.58	0.75
Hypertonicity	0.85	0.08, 1.62	**0.03**	0.09	-0.20, 0.37	0.54	0.16	-0.18, 0.50	0.36	0.29	0.05, 0.52	**0.02**	1.39	0.15, 2.62	**0.03**
Hypotonicity	0.13	-0.53, 0.78	0.70	0.20	-0.04, 0.43	0.10	0.24	-0.05, 0.52	0.10	0.21	-0.01, 0.40	**0.04**	0.76	-0.28, 1.80	0.15
Asymmetric reflexes	-0.18	-0.66, 0.30	0.47	-0.08	-0.26, 0.09	0.33	0.11	-0.10, 0.32	0.30	-0.02	-0.17, 0.13	0.81	-0.17	-0.94, 0.60	0.67
Excitability	0.16	-0.09, 0.42	0.21	0.07	-0.02, 0.16	0.12	0.06	-0.05, 0.17	0.29	0	-0.08, 0.08	0.97	0.29	-0.11, 0.70	0.16
Lethargy	0.23	0.02, 0.44	**0.04**	0.02	-0.05, 0.10	0.55	0.08	-0.02, 0.17	0.11	0.01	-0.06, 0.07	0.85	0.33	-0.01, 0.67	0.05
TIMP															
z-score	-1.12	-1.79, -0.46	**<0.01**	-0.12	-0.37, 0.13	0.34	-0.41	-0.71, -0.12	**<0.01**	-0.19	-0.40, 0.03	0.09	-1.85	-2.91, -0.78	**<0.01**
Visual n=100															
Total	-0.02	-0.10, 0.05	0.53	0	-0.03, 0.02	0.79	-0.03	-0.07, 0	0.05	0.01	-0.01, 0.03	0.31	-0.05	-0.17, 0.07	0.43

Key: Term MRI, 40-42 weeks postmenstrual age (range 38-42 weeks); WM, white matter; GM, gray matter; GMs, General Movements Assessment; HNNE, Hammersmith Neonatal Neurological Examination; NNNS, NICU Neonatal Neurobehavioral Scale; TIMP, Test of Infant Motor Performance; CI, confidence interval; ref, reference level.

References

1. Anderson PJ, Cheong JL, Thompson DK. The predictive validity of neonatal MRI for neurodevelopmental outcome in very preterm children. *Semin Perinatol* 2015; **39**: 147-58.

2. Van't Hooft J, van der Lee JH, Opmeer BC, et al. Predicting developmental outcomes in premature infants by term equivalent MRI: systematic review and meta-analysis. *Syst Rev* 2015; **4**: 71.

3. Spittle AJ, Doyle LW, Boyd RN. A systematic review of the clinimetric properties of neuromotor assessments for preterm infants during the first year of life. *Dev Med Child Neurol* 2008; **50**: 254-66.

4. Noble Y, Boyd R. Neonatal assessments for the preterm infant up to 4 months corrected age: a systematic review. *Dev Med Child Neurol* 2012; **54**: 129-39.

5. Brown NC, Inder TE, Bear MJ, Hunt RW, Anderson PJ, Doyle LW. Neurobehavior at term and white and gray matter abnormalities in very preterm infants. *J Pediatr* 2009; **155**: 32-8, 8 e1.

6. Woodward LJ, Mogridge N, Wells SW, Inder TE. Can neurobehavioral examination predict the presence of cerebral injury in the very low birth weight infant? *J Dev Behav Pediatr* 2004; **25**: 326-34.

7. Pineda RG, Tjoeng TH, Vavasseur C, Kidokoro H, Neil JJ, Inder T. Patterns of altered neurobehavior in preterm infants within the neonatal intensive care unit. *J Pediatr* 2013; **162**: 470-6 e1.

8. Spittle AJ, Brown NC, Doyle LW, et al. Quality of general movements is related to white matter pathology in very preterm infants. *Pediatrics* 2008; **121**: e1184-9.

9. Miller SP, Ferriero DM, Leonard C, et al. Early brain injury in premature newborns detected with magnetic resonance imaging is associated with adverse early neurodevelopmental outcome. *J Pediatr* 2005; **147**: 609-16.

10. Sie LT, Hart AA, van Hof J, et al. Predictive value of neonatal MRI with respect to late MRI findings and clinical outcome. A study in infants with periventricular densities on neonatal ultrasound. *Neuropediatrics* 2005; **36**: 78-89.

11. George JM, Fiori S, Fripp J, et al. Validation of an MRI Brain Injury and Growth Scoring System in Very Preterm Infants Scanned at 29- to 35-Week Postmenstrual Age. *AJNR Am J Neuroradiol* 2017: May 18. doi: 0.3174/ajnr.A5191 [Epub ahead of print].

12. Kidokoro H, Neil JJ, Inder TE. New MR imaging assessment tool to define brain abnormalities in very preterm infants at term. *AJNR Am J Neuroradiol* 2013; **34**: 2208-14.

13. Lee I, Neil JJ, Huettner PC, et al. The impact of prenatal and neonatal infection on neurodevelopmental outcomes in very preterm infants. *J Perinatol* 2014; **34**: 741-7.

14. George JM, Boyd RN, Colditz PB, et al. PPREMO: a prospective cohort study of preterm infant brain structure and function to predict neurodevelopmental outcome. *BMC Pediatr* 2015; **15**: 123.

15. Einspieler C. Prechtl's method on the qualitative assessment of general movements in preterm, term and young infants. Cambridge, UK: Mac Keith Press; 2004.

16. Darsaklis V, Snider LM, Majnemer A, Mazer B. Predictive validity of Prechtl's Method on the Qualitative Assessment of General Movements: a systematic review of the evidence. *Dev Med Child Neurol* 2011; **53**: 896-906.

17. Dubowitz L, Mercuri E, Dubowitz V. An optimality score for the neurologic examination of the term newborn. *J Pediatr* 1998; **133**: 406-16.

18. Bosanquet M, Copeland L, Ware R, Boyd R. A systematic review of tests to predict cerebral palsy in young children. *Dev Med Child Neurol* 2013; **55**: 418-26.

19. Lester BM, Tronick EZ. History and description of the Neonatal Intensive Care Unit Network Neurobehavioral Scale. *Pediatrics* 2004; **113**: 634-40.

20. El-Dib M, Massaro AN, Glass P, Aly H. Neurobehavioral assessment as a predictor of neurodevelopmental outcome in preterm infants. *J Perinatol* 2012; **32**: 299-303.

21. Stephens BE, Liu J, Lester B, et al. Neurobehavioral assessment predicts motor outcome in preterm infants. *J Pediatr* 2010; **156**: 366-71.

22. Liu J, Bann C, Lester B, et al. Neonatal neurobehavior predicts medical and behavioral outcome. *Pediatrics* 2010; **125**: e90-8.

23. Daily DK, Ellison PH. The premie-neuro: a clinical neurologic examination of premature infants. *Neonatal Netw* 2005; **24**: 15-22.

24. Ricci D, Romeo DM, Serrao F, et al. Application of a neonatal assessment of visual function in a population of low risk full-term newborn. *Early Hum Dev* 2008; **84**: 277-80.

25. Ricci D, Romeo DM, Gallini F, et al. Early visual assessment in preterm infants with and without brain lesions: correlation with visual and neurodevelopmental outcome at 12 months. *Early Hum Dev* 2011; **87**: 177-82.

26. Campbell SK, Hedeker D. Validity of the Test of Infant Motor Performance for discriminating among infants with varying risk for poor motor outcome. *J Pediatr* 2001; **139**: 546-51.

27. Flegel J, Kolobe TH. Predictive validity of the test of infant motor performance as measured by the Bruininks-Oseretsky test of motor proficiency at school age. *Phys Ther* 2002; **82**: 762-71.

28. Hack M, Breslau N, Aram D, Weissman B, Klein N, Borawski-Clark E. The effect of very low birth weight and social risk on neurocognitive abilities at school age. *J Dev Behav Pediatr* 1992; **13**: 412-20.

29. Roberts G, Howard K, Spittle AJ, Brown NC, Anderson PJ, Doyle LW. Rates of early intervention services in very preterm children with developmental disabilities at age 2 years. *J Paediatr Child Health* 2008; **44**: 276-80.

30. Stoodley CJ, Limperopoulos C. Structure-function relationships in the developing cerebellum: Evidence from early-life cerebellar injury and neurodevelopmental disorders. *Semin Fetal Neonatal Med* 2016; **21**: 356-64.

31. Clouchoux C, Guizard N, Evans AC, du Plessis AJ, Limperopoulos C. Normative fetal brain growth by quantitative in vivo magnetic resonance imaging. *Am J Obstet Gynecol* 2012; **206**: 173.e1-8.

32. Woodward LJ, Anderson PJ, Austin NC, Howard K, Inder TE. Neonatal MRI to predict neurodevelopmental outcomes in preterm infants. *N Engl J Med* 2006; **355**: 685-94.

33. Peyton C, Yang E, Kocherginsky M, et al. Relationship between white matter pathology and performance on the General Movement Assessment and the Test of Infant Motor Performance in very preterm infants. *Early Hum Dev* 2016; **95**: 23-7.

5.3 Summary of Chapter 5

This paper found structure-function relationships between MRI and concurrent clinical measures of motor, neurological and neurobehavioural function at both Early and Term MRI. At Early MRI, cerebellar subscale scores demonstrated the strongest associations with clinical measures. Early MRI cerebellar scores were related to neurological rather than neurobehavioural or motor items in the clinical measures. At Term MRI, the strongest associations were with the TIMP. White matter abnormality scores were related to motor and neurological performance at Term but not at Early MRI.

Having determined validity and diagnostic accuracy of the Early structural MRI scoring system and elucidated the associations with concurrent clinical features, the next step was to examine brain microstructural development with MR diffusion imaging.

Chapter 6: Very early brain microstructure measured with MR diffusion imaging at 32 and 40 weeks postmenstrual age in relation to 12-month motor outcome in very preterm born infants

6.1 Introduction to Chapter 6

Qualitative evaluation of structural images demonstrates that motor and cognitive outcomes can be determined earlier than TEA. The structural MRI scoring system developed in this thesis is valid and reproducible but has limitations, which include relatively low sensitivity. Advanced diffusion imaging is able to quantify microstructural development, and so the next step was to evaluate diffusion measures of fractional anisotropy (FA) and mean diffusivity (MD) in regions known to be involved in motor performance and determine their associations with 12 month outcomes.

Results from the systematic review in Chapter 2 guided the selection of brain regions for examination at Early MRI. This chapter presents results of preliminary analyses of the relationships between diffusion measures of FA and MD in the corpus callosum, posterior limb of the internal capsule (PLIC) and cerebral peduncles, with motor outcomes at 12 months corrected age. It is written as a manuscript so as to be consistent with previous chapters.

Very early brain microstructure measured with MR diffusion imaging at 32 and 40 weeks postmenstrual age in relation to 12-month motor outcome in very preterm born infants.

George JM, Fripp J, Pannek K, Shen K, Ware RS, Rose SE, Colditz PB, Boyd RN

Abstract

Aim The aim of this study was to examine brain microstructure using diffusion MRI at 30-32 weeks postmenstrual age ('Early MRI", PMA) and 40-42 weeks PMA ('Term MRI') in brain regions known to be associated with motor function. The relationship with motor and neurological outcomes at 12 months corrected age was evaluated.

Method Infants born <31 weeks' gestational age in this prospective cohort study underwent Early and Term MRI at 3T. A reference sample of healthy term-born infants underwent MRI at 40-42weeks PMA. Brain tissue microstructure was analyzed globally and in 3 regions of interest for motor function defined on the JHU neonate atlas (automatic segmentation): the posterior limb of the internal capsule, corpus callosum and cerebral peduncle. Infants with significant structural brain lesions were excluded due to challenges in automated segmentation and processing. Regional fractional anisotropy (FA) and mean diffusivity (MD) were calculated. At 12 months corrected age, preterm infants were assessed with the Bayley Scales of Infant and Toddler Development 3rd edition (Bayley III), the Alberta Infant Motor Scale (AIMS), the Neurosensory Motor Developmental Assessment (NSMDA) and a structured neurological assessment by a paediatrician. Univariable and multivariable regression was employed to examine associations between preterm infant FA and MD at Early and Term MRI, and 12 month motor outcomes. Unpaired t-tests were used to compare FA and MD of the preterm infant Term MRI with that of the reference sample.

Results Forty-eight preterm infants (born at median gestation 28^{+5} weeks, 30 male) with Early MRI at median 32^{+3}weeks PMA had useable diffusion images and 12 month outcome data available. Sixty-five preterm infants (born at median gestation 28^{+4} weeks, 39 male) with Term MRI at a median 40^{+5} weeks PMA had useable diffusion images and 12 month outcome data available. Outcomes were assessed at a mean 12 months and 4 days corrected age. Eighteen term born infants made up the reference sample (median gestational age at birth of 39^{+2} weeks, median PMA at MRI 41 weeks, 9 males). Early MRI FA and MD were not associated with any of the motor or neurological outcomes. Term MRI FA was not associated with motor or neurological outcomes. Term MRI MD in the corpus callosum on the left was associated with neurological outcome and MD in the right cerebral peduncle was associated with motor outcome on the AIMS and NSMDA. Corpus callosum FA and MD was significantly different between the preterm infants imaged at Term, and the term reference sample.

Interpretation Early MRI microstructure in this very preterm cohort without significant brain lesions was not associated with motor or neurological outcomes at 12 months corrected age. Future work may require manual segmentation of diffusion images in infants in this cohort who had significant structural brain lesions if the full representative cohort is to be included in analyses.

Introduction

Preterm infants are at risk of adverse motor outcomes including cerebral palsy and developmental co-ordination disorder [1]. Neuroimaging has improved identification of infants at risk of adverse motor development and prognostication of outcomes[2]. Evaluation of brain macrostructure on structural MRI offers qualitative information of brain injury and impaired brain growth[3-8]. Diffusion MRI provides evaluation of brain tissue microstructure and is increasingly used in studies measuring preterm brain development[9]. It involves mapping the random motion of water particles in brain tissue, and the degree to which it is hindered and restricted by tissue microstructure [10].

Quantitative diffusion measures of fractional anisotropy (degree of anisotropy, FA) and mean diffusivity (overall diffusivity, MD) demonstrate changes from preterm birth to term equivalent age[11-14] and show promise for use as biomarkers to predict later motor outcomes[9]. Brain maturation results in an increased fibre density, greater fibre organisation and increased myelination of fibers in white matter, all of which restrict water diffusion and increase the primary directionality (i.e. increase FA) and decrease overall diffusivity (i.e. decrease MD)[15]. Diffusion MRI studies of preterm infants shortly after birth and again at term equivalent age show an increase in FA and a decrease in MD over this period[11-14, 16].

Diffusion MRI measures at term equivalent age are reported to be associated with poorer motor outcome and a diagnosis of cerebral palsy at 18-24 months corrected age (CA); reduced FA and increased MD in the posterior limb of the internal capsule (PLIC) [17-19], splenium [19] and the corona radiata [20], higher radial diffusivity in the corpus callosum, fornix, internal and external capsule [21, 22], and increased MD in the cerebellum [23]. Earlier imaging studies report that reduced FA as early as 30 weeks PMA has been associated with poorer motor outcomes [24-26].

The aim of this study was to evaluate brain microstructure on Early and Term MRI in regions known to be involved in motor function, the posterior limb of the internal capsule (PLIC), corpus callosum and cerebral peduncle, and then to examine the association of these early microstructural measures with motor outcome at 12 months CA. A secondary aim was to compare preterm brain

microstructure in the 3 defined regions at Term MRI with a term born reference sample imaged at approximately 40-42 weeks PMA.

Methods

Study Design and Participants

Recruitment was conducted at the Royal Brisbane and Women's Hospital between February 2013 and April 2016 as part of the PPREMO study[27]. Infants were eligible if they were born <31 weeks gestational age, without chromosomal abnormality and with English speaking families/caregivers who lived within a 200km radius of the hospital. A reference sample of infants born between 38-41 weeks gestational age, with a birthweight >10th percentile following an uncomplicated pregnancy, delivery and postpartum period, were recruited. Informed parental consent was obtained for all participants. Ethical approval was obtained from the RBWH Human Research Ethics Committee (HREC/12/QRBW/245), The University of Queensland (2012001060) and the trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12613000280707).

MRI Acquisition

Brain MRI was performed during natural sleep and without sedation at 30-32 weeks PMA ('Early MRI'), or when the infant was sufficiently medically stable to allow safe transport to the MRI (range 29-35 weeks PMA). A second MRI was performed at 40-42 weeks PMA ('Term MRI'). Infants were scanned using a 3T Siemens Tim Trio (Erlangen, Germany), utilizing a MR compatible incubator with dedicated neonatal head coil (LMT Lammers Medical Technology, Lübeck, Germany). The term born reference sample was imaged at 40-42 weeks PMA and at least 1 week post birth. Diffusion MRI data were acquired along 64 non-collinear directions, using a b-value of 2000 s/mm^2, along with one minimally diffusion weighted image (b=0). Acquisition parameters were: TR/TE 9500/130 ms, flip angle 90°, field of view 224x224mm, matrix 128x128, 47 slices, slice thickness 2 mm, iPAT 2. A field map to assist in the correction of susceptibility distortions was acquired using a Gradient Recalled Echo sequence. Scans with significant motion were repeated if time was available.

Diffusion analysis

Diffusion data were preprocessed using previously established protocols [28, 29]. In brief, volumes containing within-volume motion artefacts were automatically detected using a registration based approach[30], and removed from analysis. Susceptibility distortions were corrected using the field map, using tools available in FSL, with adjustment of signal intensities [31, 32]. Head motion between volumes was corrected using rigid-body registration, with adjustment of the b-matrix. Signal

intensity outliers (e.g. due to bulk head motion or cardiac pulsation) were automatically detected [33] and replaced in the raw image space prior to resampling [34]. Intensity homogeneities were reduced using a bias field correction method [35]. Diffusion tensors were estimated from the corrected diffusion data using the iteratively reweighted least squares method, and measures of FA and MD were calculated.

A study specific brain MRI template was created separately for the Early and Term MRI time points. This was implemented using an in-house implementation of the Advanced Normalization Tools – Symmetric Normalization - Groupwise (ANTs-SyN-GW) extension of tract-based spatial statistics [36]. It was performed independently on Early (N=78) and Term MRI data (N =90 which included Term MRI and term reference group data). In short, this involves the creation of a groupwise population specific template using the ANTS registration framework[37]. The importance of this groupwise alignment has been highlighted[38], and involves the iterative groupwise coregistration of the images by alternating between registering each image to a shape-based mean of the inputs and recomputing this target as the mean over the coregistered set[38]. The generated template is usually the same resolution and voxel space as the original inputs and can be used as a registration target for voxel based analysis or tract-based spatial statistics. In this work we specified a higher resolution template space (2x) to more closely match the resolution of the JHU-SS-neonate atlas (0.6 mm isotropic). The ANTs-SyN-GW template was non-linearly aligned with the Johns Hopkins University (JHU) single subject FA neonate atlas[39] using ANTs (symmetric diffeomorphic registration[37]) and the atlas labels propagated onto the template. Example images of the FA template with JHU labelling at Early MRI is presented in Figure 1.

Each infant's diffusion images were then registered with the population template and the JHU labeling was resampled into the infant space after composing the transforms. White matter microstructure was then defined by the JHU labeling, with threshold values of MD < 0.006 mm^2/s and FA > 0.15. The use of this threshold in WM on near term and term equivalent neonate DTI data is well accepted[28, 40, 41] and balances the inclusion of less mature WM regions, which may better represent progression of development during the neonatal period, while minimizing inclusion of gray matter and partial volume effects. The JHU labelling was qualitatively scored (unusable, poor and good) with subjects with poor labelling defined as moderate mislabeling (> 10% of boundary with errors) along major tracts (e.g. corpus callosum, PLIC). Unusable data occurred from complete or significant failure of the registration; typically, this occurred in a subset of subjects with significant structural brain lesions.

Motor Outcome at 12 months CA

At 12 months CA motor outcome was evaluated using the Bayley Scales of Infant and Toddler Development 3rd edition (Bayley III), the Alberta Infant Motor Scale (AIMS) and the Neurosensory Motor Developmental Assessment (NSMDA). The assessments were conducted by an experienced pediatric physiotherapist, blinded to Early and Term MRI findings and clinical history. This combination of motor assessments was selected because although the Bayley III is the outcome assessment of choice for preterm follow up studies, the motor subscale demonstrates only moderate predictive validity for later motor outcomes[42]. The AIMS is a gross motor assessment with good predictive validity[43] and concurrent validity with the Bayley II[44]. The NSMDA assesses gross and fine motor performance as well as balance, posture, neurological performance and sensory motor function. At 12 months CA it demonstrates good predictive validity for school age motor and cognitive outcomes[45] and CP at 5 years[46]. The AIMS and NSMDA have been recommended for use at 12 months CA in a clinimetric review of neuromotor measures for use in the first year following preterm birth[47].

Neurological outcome at 12 months CA

A structured neurological assessment was conducted by a pediatrician, specialized in early infant development and neurological evaluation and blinded to Early and Term MRI findings. The neurological outcome was summarized as 'normal' (entirely normal neurological examination), 'unspecified signs' (e.g. hypotonia, asymmetric reflexes) or 'abnormal' (definitely abnormal neurological presentation/possible cerebral palsy) [Thesis appendix 10].

Statistical Analysis

To investigate the associations between Early FA and MD in each brain region and 12-month motor outcome, univariate linear regression was performed, followed by multivariate analysis adjusting for sex, and PMA at MRI. Corrected age at 12-month assessment was included as a covariate for analyses with the AIMS and NSMDA. To investigate the associations between Early MRI measures and neurological outcome, univariate logistic regression was performed followed by multivariate analysis adjusting for sex, PMA at Early MRI and corrected age at 12-month assessment. This was replicated to examine the associations between the Term MRI and 12-month motor and neurological outcomes. Results are presented as regression coefficients with 95% confidence intervals and the level of significance was set at 5%. Brain microstructure in the 3 regions in preterm infants imaged at Term age was compared with the term reference sample using un-paired t-tests. Analysis was performed using the Stata statistical software package, version 14 (StataCorp, College Station, TX, USA).

Results

Of 323 eligible preterm infants, 146 were recruited to the study. Diffusion MRI was acquired Early in 114 infants, and at Term in 98 infants. Early MRI with diffusion measures of sufficient quality in the brain regions of interest and 12 month outcome was available for 48 infants (42%); 48 (42%) were excluded due to motion, spikes or other artefact; 11 (10%) were excluded due to failure during diffusion processing and 7 (6%) were excluded due to failure of the automatic segmentation process. Term MRI with diffusion measures of sufficient quality in the brain regions of interest and 12 month outcome was available for 65 infants (66%); 6 (6%) were excluded due to motion, spikes or other artefact; 16 (16%) were excluded due to failure during diffusion processing and 11 (11%) were excluded due to failure of the automatic segmentation process. Thirty-one term born infants were recruited as a reference sample, of whom 18 infants had diffusion data of sufficient quality for analysis (58%). Demographic and perinatal details are summarized in Table 1; summary data of the motor and neurological outcomes for the preterm infants are presented in Table 2. Detail of numbers of study participants with diffusion MRI acquired and reasons for exclusion from analyses are detailed in Table 3. Summary data of FA and MD for the specified regions are presented in Table 4.

Associations between Early MRI microstructure and 12-month motor outcome

Results of univariable and multivariable regression analyses between Early MRI FA and 12 month outcomes are presented in the coefficient plot in Figure 2. Early MRI MD and 12 month outcomes are presented in Figure 3. No significant associations were found between FA or MD from Early MRI and any of the motor or neurological outcomes at 12 months CA on either univariable or multivariable regression.

Associations between Term MRI microstructure and 12-month motor outcome

Results of univariable and multivariable regression analyses between Term MRI FA and 12 month outcomes are presented in the coefficient plot in Figure 4. Term MRI MD and 12 month outcomes are presented in Figure 5. Term MRI FA demonstrated no associations with motor or neurological outcomes on univariable or multivariable regression. A higher MD in the left corpus callosum was associated with poorer neurological outcome both univariably and multivariably (multivariable regression coefficient β=17317; 95% confidence interval CI= 884, 33750; p=0.039). Mean diffusivity in the right cerebral peduncle was negatively associated with the AIMS (β= -88115; 95% CI= -174567, -1664; p=0.046) and NSMDA (β= -44186; 95% CI= -82843, -5529; p=0.026) on multivariable regression. Mean diffusivity in the left cerebral peduncle demonstrated a strong negative association as demonstrated in Figure 5.

Comparison of diffusion measures for preterm and term reference group at Term MRI

The results of comparisons between the Term MRI for the preterm group and the data of the term reference sample are presented in Table 5. Corpus callosum FA and MD were significantly different between the preterm infants and term infants at TEA. Left corpus callosum FA mean difference = -0.040; 95%CI=-0.055, -0.025; p<0.001. Left corpus callosum MD mean difference=0.072e-03 s/mm^2; 95%CI= 0.048e-03 s/mm^2, 0.100e-03 s/mm^2; p<0.001.

Discussion

Diffusion measures of FA and MD at Early MRI in the corpus callosum, PLIC and cerebral peduncles were not associated with motor or neurological outcomes at 12 months CA in this cohort of infants born < 31 weeks GA. Term MRI FA in the 3 regions were also not associated with motor or neurological outcomes at 12 months CA. Term MRI MD in the left corpus callosum was associated with neurological outcome; in the right cerebral peduncle it was associated with motor outcome on the AIMS and NSMDA. Corpus callosum FA and MD were significantly different between the preterm born cohort imaged at Term compared to the term born reference group.

Preterm samples at Early and Term MRI in these analyses are not representative of all infants born <31 weeks GA. Only 42% of Early MRI and 66% of Term MRI scans were useable in the present study, however this is similar to other diffusion studies in preterm infant populations[40]. Motion artefact is a challenge for all studies of diffusion MRI. In the present study, 42% of Early and 6% of Term MRI scans were excluded due to motion or other artefacts (Table 3). Failure of the automatic segmentation process occurred predominantly in cases with large structural lesions, and was responsible for exclusion of 6% of Early and 11% of Term data (Table 3). Exclusion of participants who failed the automatic segmentation process due to structural brain lesions is biasing the sample towards those with less or no structural brain injury. Manual segmentation of regions of interest is the alternative to automatic segmentation processes; however, this requires suitably skilled personnel and is time consuming and resource intensive. The benefit is that scans with distorted anatomy as a result of structural lesions can usually still be segmented and the data included in analyses. The exclusion of subjects with structural brain lesions in the present study renders this a low-risk group of very preterm infants.

Fractional anisotropy and MD in the PLIC presented here are comparable with other studies of preterm infants with diffusion imaging around 30 weeks PMA and Term[14, 39, 48]. Fractional anisotropy increased and MD decreased from Early to Term MRI in all regions studied in this preterm cohort; consistent with studies of maturational changes in diffusion measures in very

preterm infants[11, 13, 14, 16, 40]. Fractional anisotropy in the corpus callosum was higher and MD was lower in the term reference group compared to the preterm infants imaged at Term. Differences in the corpus callosum FA between term and preterm infants have been established and this data supports previous findings[22, 49, 50].

This study found no association between FA in the 3 regions studied at Early or Term MRI and motor outcomes. There are conflicting reports of Early diffusion MRI associations with motor outcome in the literature. Associations between lower FA in the PLIC at 30 weeks PMA and poorer motor outcomes have been reported in a small study of 12 participants, with manual segmentation of the PLIC and inclusion of infants with an outcome of CP[24]. A larger study (n=157) which included some MRI which were performed >36 weeks PMA, found a lower FA in the corpus callosum, PLIC and optic radiation when the cohort was grouped by motor outcome on the Bayley III[25]. Using the same cohort, but stratifying the group into 27-29, 30-33 and 34-36 weeks PMA at MRI, no associations were found between FA in the corpus callosum and motor outcomes[51]. Once again, in the same cohort, lower FA in the corpus callosum genu and splenium was found to be associated with poorer motor outcomes, but analyses pooled the Early and Term MRI data[26].

Our finding of no associations between Term MRI FA and motor outcomes contrasts with other published data which have reported a lower FA in the PLIC associated with poorer motor outcomes[17-19, 24]. Those studies had similar inclusion criteria in terms of GA at birth, one study excluded infants with structural brain lesions on conventional MRI[17], while the others did not exclude any infants with brain lesions[18, 19]. The key difference is that those studies all used manual delineation of regions of interest, not automatic segmentation as employed in the current study. Another difference between our sample and those published data is that none of the infants in our analyses had an outcome of CP, whereas their samples ranged from 5%[17]-6%[18].

An additional factor that makes Early MRI more challenging than Term MRI analyses was that sicker infants in the present study have their MRI at later PMA when they are medically stable for transport to MRI compared with stable and robust infants who are able to undergo MRI earlier. As FA increases rapidly with maturation between 30 and 40 weeks PMA, it is possible that FA in a more stable infant imaged earlier and a sick infant imaged later are similar, but if they had been imaged at the same PMA, the sicker infant (most at risk for subsequent poorer motor outcomes) may have had a lower FA. All multivariate analyses were adjusted for PMA at MRI but this may not be sufficient to account for these rapid changes. More detailed examination of this cohort

stratified by week of PMA at MRI may elucidate if this variability in PMA at MRI is clouding findings of lower FA being associated with poorer motor outcomes.

It is possible that the threshold for 'useable quality diffusion MRI data' in the present study was too strict resulting in exclusion of too much data. Future work will investigate if the threshold can be relaxed by either improving post processing methods or investigating additional statistical analysis techniques. The effect of removal of volumes on FA and MD may be less severe with 64-direction HARDI data than with traditional 30-direction DTI data, because there is an increased redundancy of data available for fitting the diffusion tensor. Future work in the current cohort will be to perform manual segmentation of the cases excluded due to failure of the automatic segmentation which was the result of significant structural brain lesions. Examination of diffusion measures such as NODDI (neurite orientation dispersion and density imaging) and FOD (fibre orientation distribution) will also be undertaken, which may be more sensitive.

Strengths of the current study include advanced diffusion imaging data acquired in 64 encoding directions and motor outcomes assessed using a combination of tools with good predictive validity (AIMs and NSMDA) in addition to the Bayley III. Limitations include an underrepresentation of infants with structural brain lesions and the fact that neurological status was assessed with a structured assessment by an experienced paediatrician, but a validated tool such as the Hammersmith Infant Neurological Examination which generates a continuous score might have been better able to detect clinically meaningful differences between infants.

Conclusion

This study found no associations between Early MRI FA or MD, and 12 month motor or neurological outcomes in this cohort of very preterm infants without significant structural brain lesions. No associations were found between Term MRI FA and 12 month outcomes; however, MD in the corpus callosum was associated with neurological outcome, and MD in the right cerebral peduncle was associated with motor outcome on the AIMS and NSMDA. Loss of data due to motion artefact is inevitable in diffusion MRI studies. Loss of data due to failure of automatic segmentation methods reduces the potential of diffusion imaging studies to answer clinically meaningful questions in representative populations of very preterm infants. Until automatic segmentation methods can handle tissue distortion from structural brain lesions, this methodology remains confined to studies of predominantly normative or low risk populations of preterm infants.

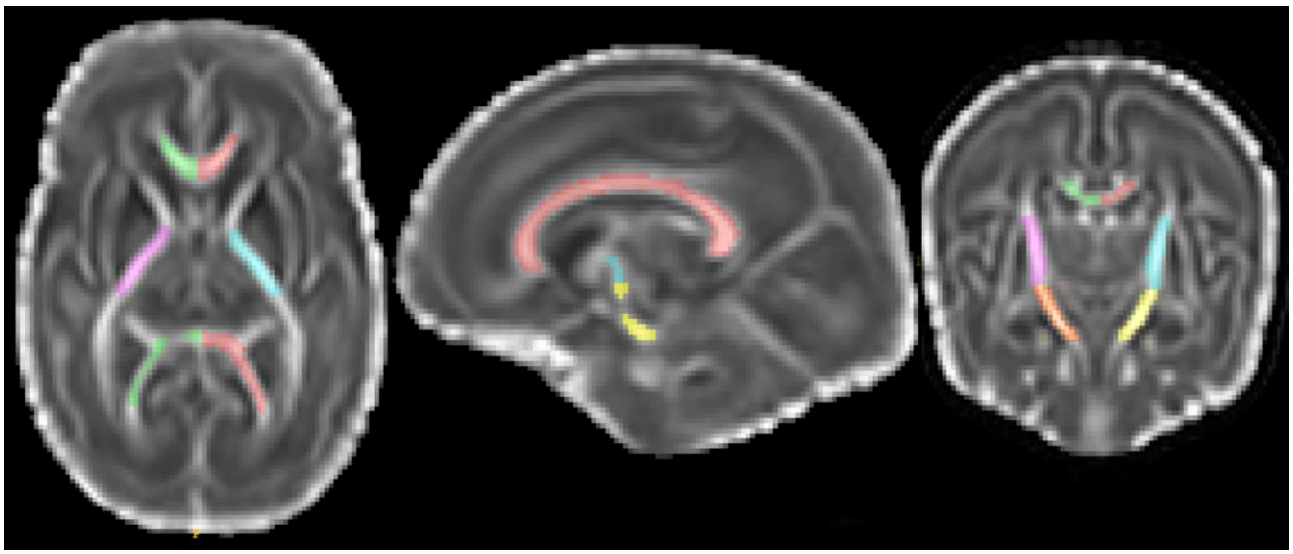


Figure 1: Early brain MRI fractional anisotropy template at 32 weeks postmenstrual age (built from n=78 preterm infant Early MRI). Regions of interest delineated by John Hopkins University labelling on (left to right) axial, sagittal and coronal images. Colours: green, right corpus callosum; red, left corpus callosum; pink, right posterior limb of the internal capsule (PLIC); blue, left PLIC; orange, right cerebral peduncle; yellow, left cerebral peduncle.

Table 1: Characteristics of the study samples included in chapter 6

	Sample with Early MRI n=48	**Sample with Term MRI n=65**	**Term Reference Sample n=18**
Birth & Maternal Data	**n(%), Median[IQR] or Mean(SD), range**		
Gestational age at birth (weeks.days)	28.5 [27.4-29.4], 23.1-30.6	28.4 [27.1-29.3], 23.1-30.6	39.2 [38.4-40], 38.2-41.3
Birth weight (g)	1152 (285), 580-1886	1090 (312), 524-1886	3386 (272), 2932-3940
Birth head circumference (cm)	26.15 (2.00), 22.50-30.50, n=44	25.74 (2.10), 21.50-30.50, n=63	34.48 (1.10), 32.50-36.40, n=17
Males	30 (63%)	39 (60%)	9 (50%)
Multiple births	12 (25%)	19 (29%)	0
Premature rupture of membranes	10 (21%)	14 (22%)	1 (6%), n=17
Caesarian section	33 (69%)	49 (75%)	5 (29%), n=17
Chorioamnionitis	4 (8%)	10 (15%)	
Antenatal steroids	35 (73%)	49(75%)	
Magnesium sulphate	25 (52%), n=39	37 (57%), n=57	
Higher social risk	19 (40%)	25 (38%)	2 (11%)
Acquired medical factors	**From birth to Early MRI**	**From birth to Term MRI**	
Patent ductus arteriosus	21 (44%)	36 (55%)	
IVH any grade	11 (23%)	7 (11%)	
IVH grade 3 or 4	4 (8%)	3 (5%)	
Periventricular leukomalacia	1 (2%)	1 (2%)	
Hydrocephalus	0	0	
NEC diagnosed or suspected	2 (4%)	1 (2%)	
Confirmed sepsis	2 (4%)	3 (5%)	
Total parenteral nutrition (days)	11.5 [6.5-14.5], 0-30	11 [9-14], 0-36	
Postnatal corticosteroids	6 (13%)	9 (14%)	
Ventilation (days)	2 [0-5.5], 0-50	2 [0-7], 0-50	
CPAP (days)	14.5 [6.5-24], 0-47	15 [7-22], 1-47	
Oxygen therapy (hours)	12 [2-82], 0-1515, n=45	33.5 [2-127], 0-1264, n=56	
O_2at 36 weeks PMA	8 (17%)	16 (25%)	
PMA at MRI (weeks.days)	32.3 [31.2-33.2], 30.2-35	40.5 [40-41.3], 38.3-42.5	41 [40.1-41.2], 39.2-42.4
Weight at MRI (g)	1545 [1308-1729], 1023-2715	3000 [2700-3375], 1900-4114	3400 [3100-3600], 2810-3880

Key: Early MRI, 29-35 weeks PMA; Term MRI, approximately 40-42 weeks PMA; PMA, postmenstrual age; IVH, Intraventricular hemorrhage; NEC, necrotizing enterocolitis; CPAP, continuous positive airway pressure.

Table 2: Summary of motor and neurological outcome data for the preterm group included in chapter 6

	Sample with Early MRI n=48	**Sample with Term MRI n=65**
Bayley III motor composite	100 [92.5-110], 67-127	97 [88-110], 52-127
AIMS total	53 [52-53.5], 31-58	53 [51-53], 16-58
NSMDA total	186 [178.5-191], 146-199	185 [174-190], 117-199
Neurological outcome:		
Normal	32 (70%)	45 (74%)
Unspecified signs	12 (26%)	16 (26%)
Definitely abnormal	2 (4%)	0

Key: Early MRI, 29-35 weeks PMA; Term MRI, approximately 40-42 weeks PMA; Bayley III Bayley Scales of Infant and Toddler Development Version III, AIMS Alberta Infant Motor Scale, NSMDA Neurosensory Motor Developmental Assessment.

Table 3: MRI acquisition numbers and reasons for exclusion from analyses in chapter 6

	Early MRI	**Term MRI**
Diffusion MRI acquired	114	98
Useable diffusion data and 12 month outcomes and included in analyses	48 (42%)	65 (66%)
Excluded due to motion (>10% of volumes), spikes or other artefact	48 (42%)	6 (6%)
Excluded due to failure of post-processing diffusion modelling	11 (10%)	16 (16%)
Excluded due to failure of automatic segmentation	7 (6%)	11 (11%)

Key: Early MRI, 29-35 weeks PMA; Term MRI, approximately 40-42 weeks PMA

Table 4: Summary of FA and MD in corpus callosum, PLIC and cerebral peduncles for infants included in chapter 6

	Preterm group Early MRI n=48		**Preterm group Term MRI n=65**		**Term reference group n=18**	
			Fractional Anisotropy			
Region	Mean	SD	Mean	SD	Mean	SD
Global	0.214	0.005	0.230	0.006	0.237	0.006
Corpus Callosum L	0.299	0.018	0.350	0.029	0.390	0.024
Corpus Callosum R	0.299	0.019	0.345	0.029	0.390	0.022
PLIC L	0.354	0.022	0.433	0.022	0.426	0.016
PLIC R	0.357	0.020	0.429	0.021	0.425	0.016
Cerebral peduncle L	0.301	0.018	0.374	0.023	0.376	0.017
Cerebral peduncle R	0.307	0.020	0.374	0.024	0.380	0.014
			Mean Diffusivity (×10E-3 s/mm^2)			
	Mean	SD	Mean	SD	Mean	SD
Global	1.296	0.023	1.232	0.034	1.202	0.039
Corpus Callosum L	1.409	0.035	1.347	0.042	1.275	0.056
Corpus Callosum R	1.416	0.036	1.360	0.046	1.297	0.051
PLIC L	1.118	0.040	0.956	0.031	0.964	0.027
PLIC R	1.122	0.042	0.971	0.035	0.968	0.025
Cerebral peduncle L	1.137	0.032	1.071	0.049	1.051	0.032
Cerebral peduncle R	1.144	0.036	1.080	0.048	1.059	0.030

Key: Early MRI, 29-35 weeks PMA; Term MRI, approximately 40-42 weeks PMA; L left; PLIC posterior limb of the internal capsule; R right; SD standard deviation

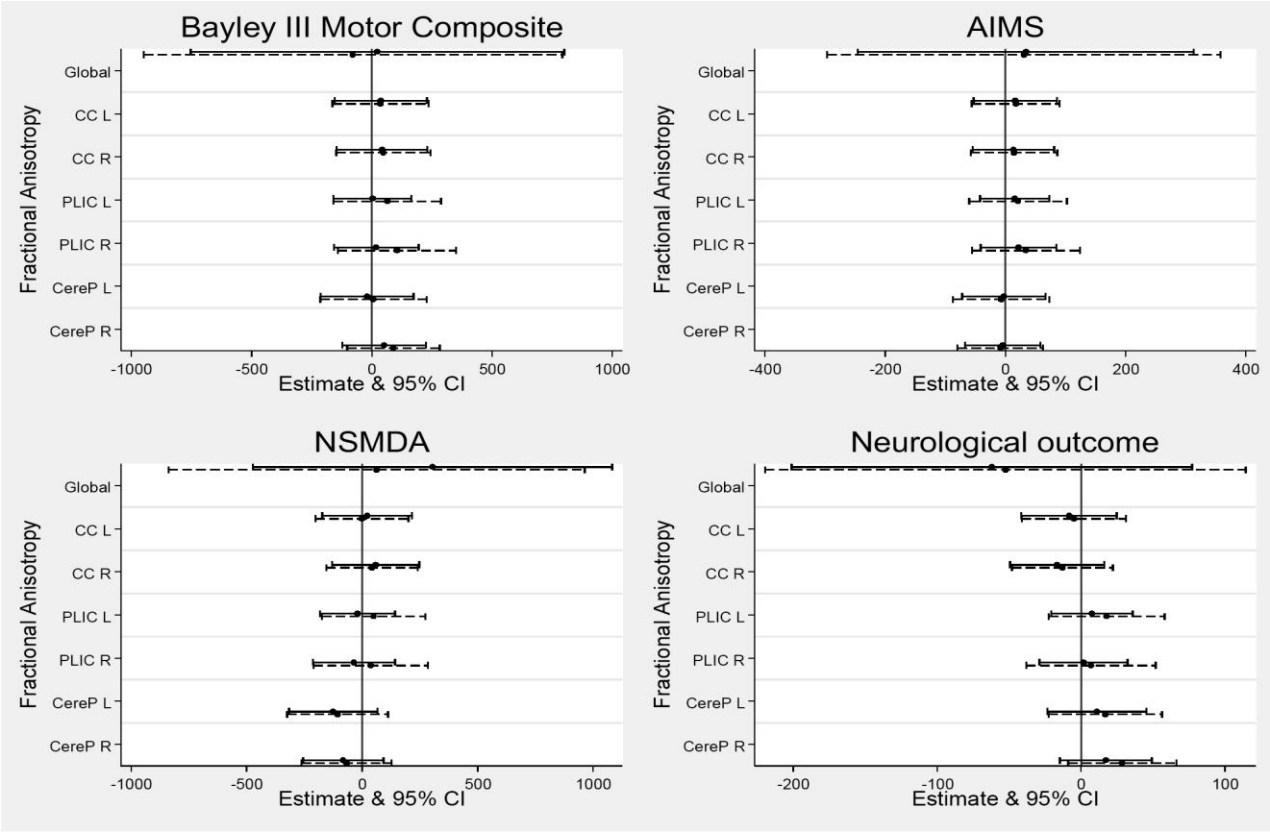


Figure 2: Coefficient plots of Early MRI fractional anisotropy and 12 month motor outcomes. Solid lines represent univariable analyses; dashed lines represent multivariable analyses.

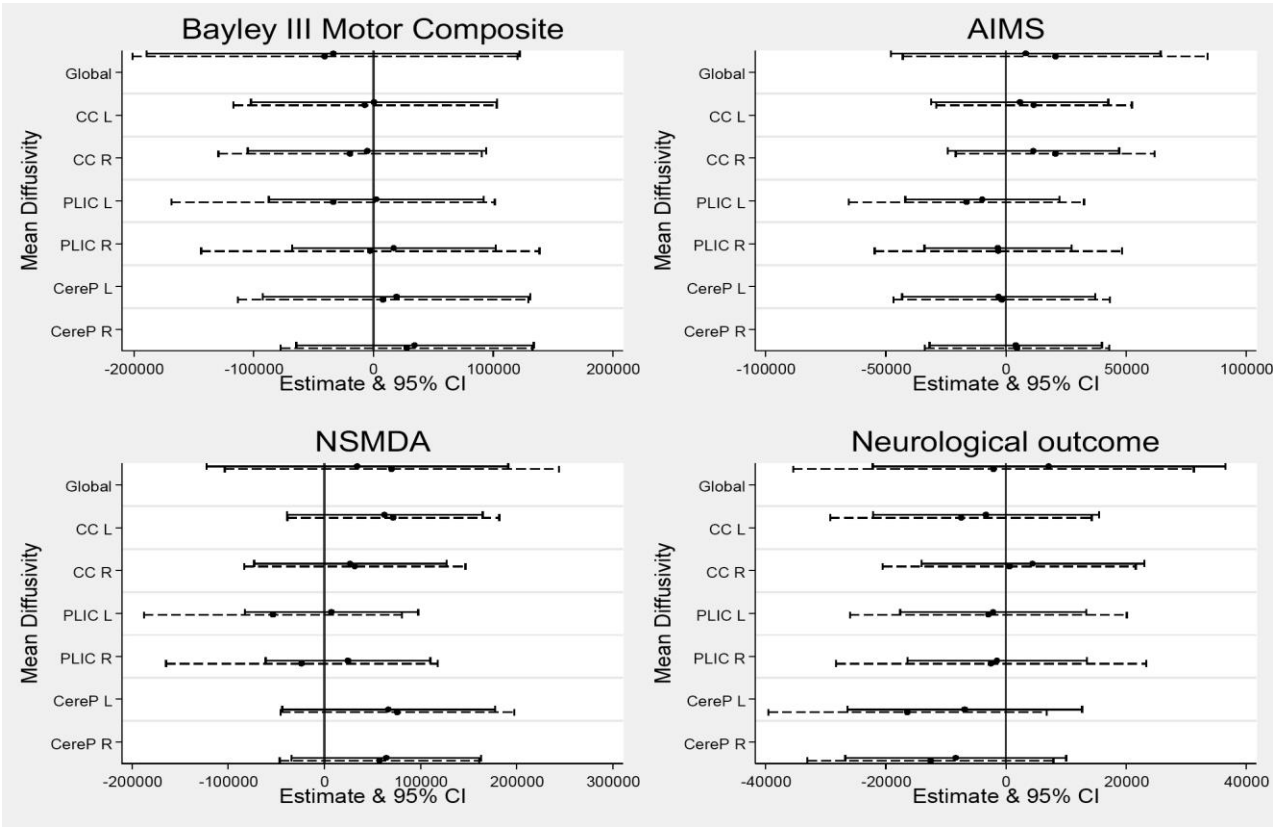


Figure 3: Coefficient plots of Early MRI mean diffusivity and 12 month motor outcomes. Solid lines represent univariable analyses; dashed lines represent multivariable analyses.

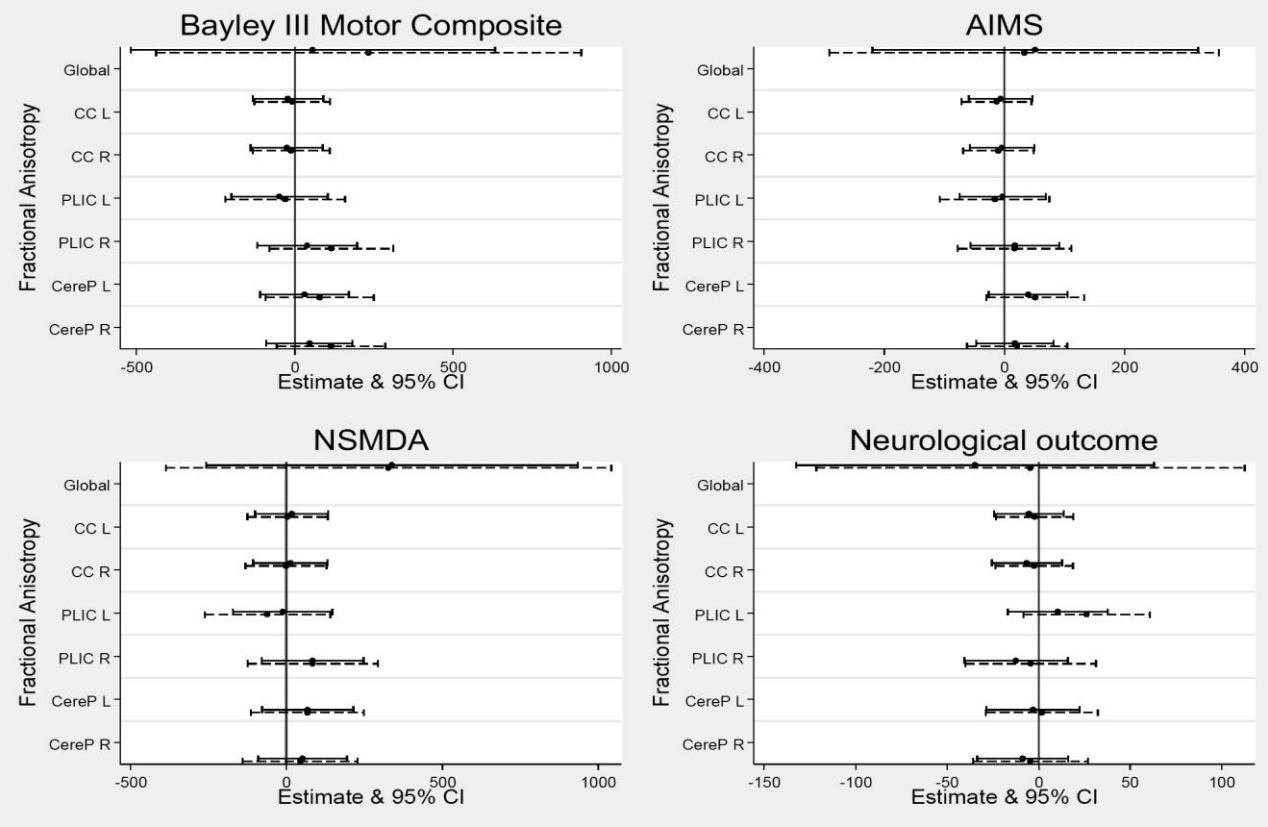


Figure 4: Coefficient plots of Term MRI fractional anisotropy and 12 month motor outcomes. Solid lines represent univariable analyses; dashed lines represent multivariable analyses.

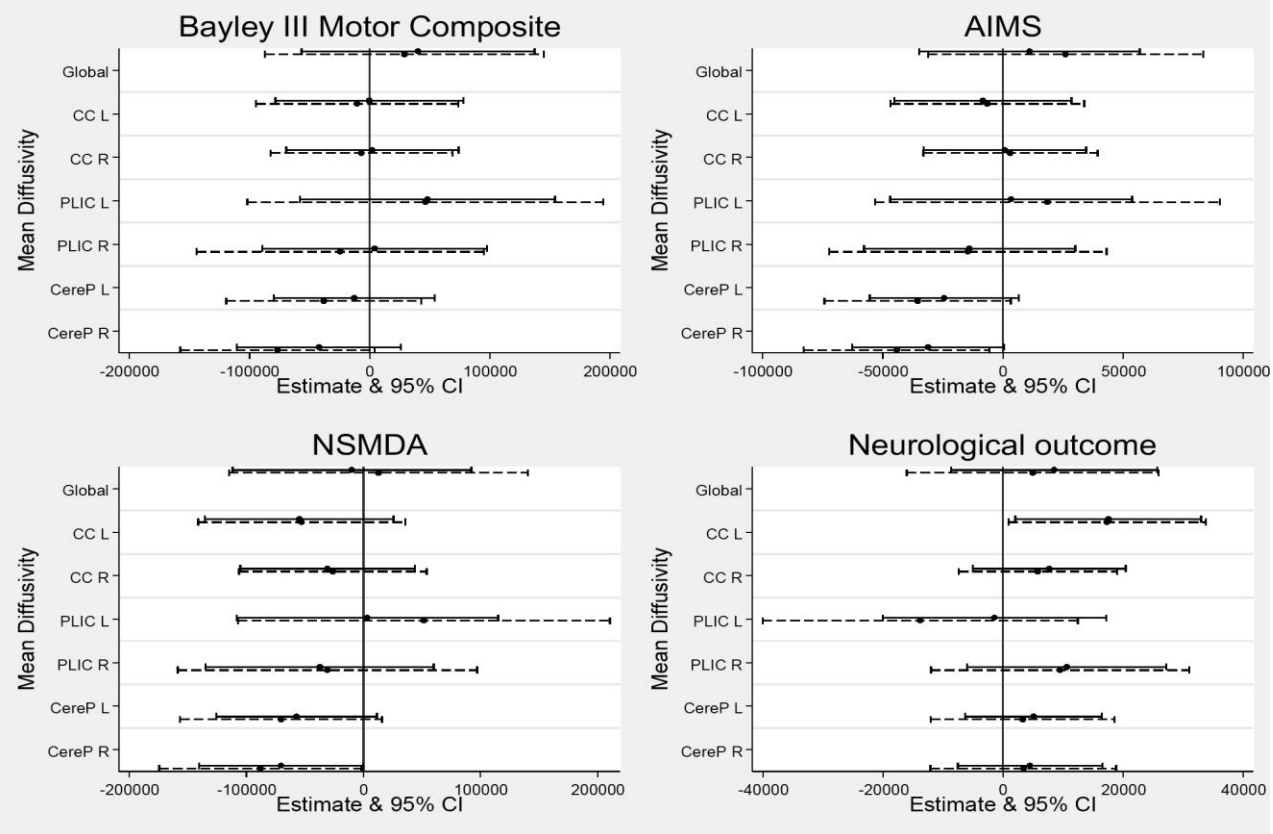


Figure 5: Coefficient plots of Term MRI mean diffusivity and 12 month motor outcomes. Solid lines represent univariable analyses; dashed lines represent multivariable analyses.

Table 5: Results of comparison between preterm infant Term MRI (n=65) and term reference group (n=18)

	Fractional Anisotropy			**Mean Diffusivity (×10E-3 s/mm^2)**		
	Mean difference	**95% CI**	**p**	**Mean difference**	**95% CI**	**p**
Global	-0.007	-0.010, -0.004	**0.000**	0.029	0.011, 0.048	**0.002**
Corpus Callosum left	-0.040	-0.055, -0.025	**0.000**	0.072	0.048, 0.100	**0.000**
Corpus Callosum right	-0.043	-0.057, -0.028	**0.000**	0.063	0.038, 0.088	**0.000**
PLIC left	0.007	-0.004, 0.018	0.207	-0.008	-0.024, 0.008	0.337
PLIC right	0.004	-0.006, 0.015	0.428	0.003	-0.015, 0.020	0.751
Cerebral peduncle left	-0.001	-0.013, 0.010	0.802	0.019	-0.005, 0.044	0.120
Cerebral peduncle right	-0.006	-0.018, 0.005	0.317	0.022	-0.002, 0.045	0.073

Key: CI confidence interval; PLIC posterior limb of the internal capsule

References

1. de Kieviet JF, Piek JP, Aarnoudse-Moens CS, Oosterlaan J. Motor development in very preterm and very low-birth-weight children from birth to adolescence: a meta-analysis. *JAMA* 2009; **302**: 2235-42.

2. Bosanquet M, Copeland L, Ware R, Boyd R. A systematic review of tests to predict cerebral palsy in young children. *Dev Med Child Neurol* 2013; **55**: 418-26.

3. Inder TE, Wells SJ, Mogridge NB, Spencer C, Volpe JJ. Defining the nature of the cerebral abnormalities in the premature infant: a qualitative magnetic resonance imaging study. *J Pediatr* 2003; **143**: 171-9.

4. Woodward LJ, Anderson PJ, Austin NC, Howard K, Inder TE. Neonatal MRI to predict neurodevelopmental outcomes in preterm infants. *N Engl J Med* 2006; **355**: 685-94.

5. Miller SP, Ferriero DM, Leonard C, et al. Early brain injury in premature newborns detected with magnetic resonance imaging is associated with adverse early neurodevelopmental outcome. *J Pediatr* 2005; **147**: 609-16.

6. Sie LT, Hart AA, van Hof J, et al. Predictive value of neonatal MRI with respect to late MRI findings and clinical outcome. A study in infants with periventricular densities on neonatal ultrasound. *Neuropediatrics* 2005; **36**: 78-89.

7. Kidokoro H, Neil JJ, Inder TE. New MR imaging assessment tool to define brain abnormalities in very preterm infants at term. *AJNR Am J Neuroradiol* 2013; **34**: 2208-14.

8. George JM, Fiori S, Fripp J, et al. Validation of an MRI Brain Injury and Growth Scoring System in Very Preterm Infants Scanned at 29- to 35-Week Postmenstrual Age. *AJNR Am J Neuroradiol* 2017: May 18. doi: 0.3174/ajnr.A5191 [Epub ahead of print].

9. Anderson PJ, Cheong JL, Thompson DK. The predictive validity of neonatal MRI for neurodevelopmental outcome in very preterm children. *Semin Perinatol* 2015; **39**: 147-58.

10. Beaulieu C. The Biological Basis of Diffusion Anisotropy. In: Johansen-Berg H, Behrens TE, editors. Diffusion MRI: from quantitative measurement to in vivo neuroanatomy: Academic Press; 2013. 105-26.

11. Huppi PS, Maier SE, Peled S, et al. Microstructural development of human newborn cerebral white matter assessed in vivo by diffusion tensor magnetic resonance imaging. *Pediatr Res* 1998; **44**: 584-90.

12. Adams E, Chau V, Poskitt KJ, Grunau RE, Synnes A, Miller SP. Tractography-based quantitation of corticospinal tract development in premature newborns. *J Pediatr* 2010; **156**: 882-8, 8 e1.

13. Ball G, Srinivasan L, Aljabar P, et al. Development of cortical microstructure in the preterm human brain. *Proc Natl Acad Sci U S A* 2013; **110**: 9541-6.

14. Schneider J, Kober T, Bickle Graz M, et al. Evolution of T1 Relaxation, ADC, and Fractional Anisotropy during Early Brain Maturation: A Serial Imaging Study on Preterm Infants. *AJNR Am J Neuroradiol* 2016; **37**: 155-62.

15. Dubois J, Dehaene-Lambertz G, Perrin M, et al. Asynchrony of the early maturation of white matter bundles in healthy infants: quantitative landmarks revealed noninvasively by diffusion tensor imaging. *Hum Brain Mapp* 2008; **29**: 14-27.

16. Kersbergen KJ, Leemans A, Groenendaal F, et al. Microstructural brain development between 30 and 40 weeks corrected age in a longitudinal cohort of extremely preterm infants. *Neuroimage* 2014; **103**: 214-24.

17. Arzoumanian Y, Mirmiran M, Barnes PD, et al. Diffusion tensor brain imaging findings at term-equivalent age may predict neurologic abnormalities in low birth weight preterm infants. *AJNR Am J Neuroradiol* 2003; **24**: 1646-53.

18. De Bruine FT, Van Wezel-Meijler G, Leijser LM, et al. Tractography of white-matter tracts in very preterm infants: a 2-year follow-up study. *Dev Med Child Neurol* 2013; **55**: 427-33.

19. Rose J, Butler EE, Lamont LE, Barnes PD, Atlas SW, Stevenson DK. Neonatal brain structure on MRI and diffusion tensor imaging, sex, and neurodevelopment in very-low-birthweight preterm children. *Dev Med Child Neurol* 2009; **51**: 526-35.

20. Kaukola T, Perhomaa M, Vainionpaa L, et al. Apparent diffusion coefficient on magnetic resonance imaging in pons and in corona radiata and relation with the neurophysiologic measurement and the outcome in very preterm infants. *Neonatology* 2010; **97**: 15-21.

21. van Kooij BJ, de Vries LS, Ball G, et al. Neonatal tract-based spatial statistics findings and outcome in preterm infants. *AJNR Am J Neuroradiol* 2012; **33**: 188-94.

22. Thompson DK, Inder TE, Faggian N, et al. Corpus callosum alterations in very preterm infants: perinatal correlates and 2 year neurodevelopmental outcomes. *Neuroimage* 2012; **59**: 3571-81.

23. Brouwer MJ, van Kooij BJ, van Haastert IC, et al. Sequential cranial ultrasound and cerebellar diffusion weighted imaging contribute to the early prognosis of neurodevelopmental outcome in preterm infants. *PLoS One* 2014; **9**: e109556.

24. Drobyshevsky A, Bregman J, Storey P, et al. Serial diffusion tensor imaging detects white matter changes that correlate with motor outcome in premature infants. *Dev Neurosci* 2007; **29**: 289-301.

25. Chau V, Synnes A, Grunau RE, Poskitt KJ, Brant R, Miller SP. Abnormal brain maturation in preterm neonates associated with adverse developmental outcomes. *Neurology* 2013; **81**: 2082-9.

26. Malavolti AM, Chau V, Brown-Lum M, et al. Association between corpus callosum development on magnetic resonance imaging and diffusion tensor imaging, and

neurodevelopmental outcome in neonates born very preterm. *Dev Med Child Neurol* 2017; **59**: 433-40.

27. George JM, Boyd RN, Colditz PB, et al. PPREMO: a prospective cohort study of preterm infant brain structure and function to predict neurodevelopmental outcome. *BMC Pediatr* 2015; **15**: 123.

28. Pannek K, Guzzetta A, Colditz PB, Rose SE. Diffusion MRI of the neonate brain: acquisition, processing and analysis techniques. *Pediatr Radiol* 2012; **42**: 1169-82.

29. Pannek K, Boyd RN, Fiori S, Guzzetta A, Rose SE. Assessment of the structural brain network reveals altered connectivity in children with unilateral cerebral palsy due to periventricular white matter lesions. *NeuroImage Clinical* 2014; **5**: 84-92.

30. Pannek K, Fripp J, George J, Boyd R, Colditz P, Rose S. Automatic detection of volumes affected by subvolume movement. International Society for Magnetic Resonance in Medicine (ISMRM); Honolulu, USA; 2017.

31. Jenkinson M. Fast, automated, N-dimensional phase-unwrapping algorithm. *Magn Reson Med* 2003; **49**: 193-7.

32. Jenkinson M. Improving the registration of B0-distorted EPI images using calculated cost function weights. Tenth International Conference on functional mapping of the human brain2004.

33. Pannek K, Raffelt D, Bell C, Mathias JL, Rose SE. HOMOR: higher order model outlier rejection for high b-value MR diffusion data. *Neuroimage* 2012; **63**: 835-42.

34. Morris D, Nossin-Manor R, Taylor MJ, Sled JG. Preterm neonatal diffusion processing using detection and replacement of outliers prior to resampling. *Magn Reson Med* 2011; **66**: 92-101.

35. Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging* 1998; **17**: 87-97.

36. Schwarz CG, Reid RI, Gunter JL, et al. Improved DTI registration allows voxel-based analysis that outperforms tract-based spatial statistics. *Neuroimage* 2014; **94**: 65-78.

37. Avants BB, Epstein CL, Grossman M, Gee JC. Symmetric diffeomorphic image registration with cross-correlation: evaluating automated labeling of elderly and neurodegenerative brain. *Med Image Anal* 2008; **12**: 26-41.

38. Keihaninejad S, Ryan NS, Malone IB, et al. The importance of group-wise registration in tract based spatial statistics study of neurodegeneration: a simulation study in Alzheimer's disease. *PLoS One* 2012; **7**: e45996.

39. Oishi K, Mori S, Donohue PK, et al. Multi-contrast human neonatal brain atlas: application to normal neonate development analysis. *Neuroimage* 2011; **56**: 8-20.

40. Rose J, Vassar R, Cahill-Rowley K, Guzman XS, Stevenson DK, Barnea-Goraly N. Brain microstructural development at near-term age in very-low-birth-weight preterm infants: an atlas-based diffusion imaging study. *Neuroimage* 2014; **86**: 244-56.

41. Vassar RL, Barnea-Goraly N, Rose J. Identification of neonatal white matter on DTI: influence of more inclusive thresholds for atlas segmentation. *PLoS One* 2014; **9**: e115426.

42. Luttikhuizen Dos Santos ES, de Kieviet JF, Konigs M, van Elburg RM, Oosterlaan J. Predictive value of the Bayley Scales of Infant Development on development of very preterm/very low birth weight children: A meta-analysis. *Early Hum Dev* 2013; **89**: 487-96.

43. Darrah J, Piper M, Watt MJ. Assessment of gross motor skills of at-risk infants: predictive validity of the Alberta Infant Motor Scale. *Dev Med Child Neurol* 1998; **40**: 485-91.

44. Jeng SF, Yau KI, Chen LC, Hsiao SF. Alberta infant motor scale: reliability and validity when used on preterm infants in Taiwan. *Phys Ther* 2000; **80**: 168-78.

45. Burns Y, O'Callaghan M, McDonell B, Rogers Y. Movement and motor development in ELBW infants at 1 year is related to cognitive and motor abilities at 4 years. *Early Hum Dev* 2004; **80**: 19-29.

46. Spittle AJ, Lee KJ, Spencer-Smith M, Lorefice LE, Anderson PJ, Doyle LW. Accuracy of Two Motor Assessments during the First Year of Life in Preterm Infants for Predicting Motor Outcome at Preschool Age. *PLoS One* 2015; **10**: e0125854.

47. Spittle AJ, Doyle LW, Boyd RN. A systematic review of the clinimetric properties of neuromotor assessments for preterm infants during the first year of life. *Dev Med Child Neurol* 2008; **50**: 254-66.

48. Nossin-Manor R, Card D, Morris D, et al. Quantitative MRI in the very preterm brain: assessing tissue organization and myelination using magnetization transfer, diffusion tensor and T(1) imaging. *Neuroimage* 2013; **64**: 505-16.

49. Rose SE, Hatzigeorgiou X, Strudwick MW, Durbridge G, Davies PS, Colditz PB. Altered white matter diffusion anisotropy in normal and preterm infants at term-equivalent age. *Magn Reson Med* 2008; **60**: 761-7.

50. Li K, Sun Z, Han Y, Gao L, Yuan L, Zeng D. Fractional anisotropy alterations in individuals born preterm: a diffusion tensor imaging meta-analysis. *Dev Med Child Neurol* 2015; **57**: 328-38.

51. Duerden EG, Foong J, Chau V, et al. Tract-based spatial statistics in preterm-born neonates predicts cognitive and motor outcomes at 18 months. *AJNR Am J Neuroradiol* 2015; **36**: 1565-71.

6.2 Summary of Chapter 6

This chapter reported results of diffusion measures of FA and MD in the corpus callosum, PLIC and cerebral peduncle on Early and Term MRI, and the relationship with 12 month motor and neurological outcomes. Early MRI FA and MD were not associated with either motor or neurological outcomes. Term MRI FA was not associated with motor or neurological outcome. Term MRI MD in the left corpus callosum was associated with neurological outcome and MD in the right cerebral peduncle was associated with motor outcome on the AIMS and NSMDA. Corpus callosum FA and MD were significantly different between the preterm born group imaged at Term compared to the term born reference group.

Although not presented in this thesis, further examination of our diffusion data found similarities with other studies of low risk cohorts, in particular that by Rose *et al*, 2014[27]. Our data at a median PMA 32 weeks was consistent with their data at near-term and TEA, with a regional pattern of higher FA and lower MD suggestive of more advanced development in posterior compared to anterior regions of subcortical WM structures. A relationship in WM with PMA at MRI was found, but limited evidence of an effect of GA at birth.

Infants with significant structural brain lesions were excluded from analyses with diffusion MRI data in chapter 6, biasing the sample towards those with less or no structural brain injury. To examine the similarities and differences between the sample included in the diffusion analyses compared to the overall recruited cohort and the samples included in papers 3 and 4, the following tables are presented: Supplementary Table 1 presents perinatal and medical characteristics of each sample; Supplementary Table 2 presents 12 month outcome data.

For the birth and maternal data, analyses were conducted to determine any statistically significant differences between each paper/chapter's sample and the infants recruited but not included in that analysis. For example, 146 infants were recruited to the study, of whom 119 underwent Early MRI and concurrent clinical assessment and are included in the analyses in paper 4. Statistical analysis compared these 119 included infants with the 27 infants recruited but without Early MRI. A discussion of the data presented in Supplementary Tables 1 and 2 follows the tables.

Supplementary Table 1: Perinatal and medical characteristics of the overall PPREMO cohort, followed by each of the samples included in the analyses for each paper/chapter presented in this thesis

	Recruited	**With Early MRI & clinical correlates** (paper 4; chapter 5)	**sMRI validation sample** (paper 3; chapter 4)	**Number included in Early DWI analysis** (chapter 6)	**Number included in Term DWI analysis** (chapter 6)
Number	146	119	83	48	65
Birth & Maternal Data					
Gestational age at birth (weeks.days)	28.1 [26.2-29.2], 23-30.6	28.3 [26.6-29.3], 23.1-30.6*	28.4 [26.6-29.3], 23.6-30.6	28.5 [27.4-29.4], 23.1-30.6*	28.4 [27.1-29.3], 23.1-30.6
Birth weight (g)	1066 (317), 494-1886	1093 (321), 494-1886*	1069 (312), 494-1886	1152 (285), 580-1886*	1090 (312), 524-1886
Birth head circumference (cm)	25.57 (2.40), 20.50-30.50 n=139	25.77 (2.36), 20.50-30.50* n=114	25.62 (2.38), 20.50-30.50 n=80	26.15 (2.00), 22.50-30.50 n=44	25.74 (2.10), 21.50-30.50 n=63
Males	91 (62%)	73 (61%)	49 (59%)	30 (63%)	39 (60%)
Multiple births	39 (27%)	36 (30%)*	24 (29%)	12 (25%)	19 (29%)
Premature rupture of membranes	37 (26%)	27 (23%)	19 (23%)	10 (21%)	14 (22%)
Caesarean section	96 (66%)	84 (71%)*	60 (72%)	33 (69%)	49 (75%)*
Chorioamnionitis	26 (18%)	18 (15%)	14 (17%)	4 (8%)*	10 (15%)
Antenatal steroids	102 (70%), n=145	83 (70%)	62 (75%)	35 (73%)	49(75%)
Magnesium sulphate	76 (52%), n=113	63 (64%), n=98	43 (52%), n=66	25 (52%), n=39	37 (57%), n=57
Higher social risk	71 (53%), n=134	58 (49%), n=118*	40 (48%)	19 (40%)*	25 (38%)*
Acquired medical factors					
Patent ductus arteriosus		59 (50%)	39 (47%)	21 (44%)	36 (55%)
IVH		30 (25%)	17 (20%)	11 (23%)	7 (11%)
IVH grade 3 or 4		8 (7%)	4 (5%)	4 (8%)	3 (5%)
Periventricular leukomalacia		4 (3%)	2 (2%)	3 (6%)	3 (5%)
Hydrocephalus		4 (3%)	2 (2%)	0	0
NEC diagnosed or suspected		5 (4%)	3 (4%)	2 (4%)	1 (2%)
Confirmed sepsis		5 (4%)	3 (4%)	2 (4%)	3 (5%)
Anti-convulsant treatment		1 (1%)	1 (1%)	0	0
Postnatal corticosteroids		20 (17%)	14 (17%)	6 (13%)	9 (14%)

Total parenteral nutrition (days)	11 [7-14], 0-36	11 [8-14], 0-30	11.5 [6.5-14.5], 0-30	11 [9-14], 0-36
Ventilation (days)	2 [0-10], 0-50, n=118	3 [0-12], 0-48	2 [0-5.5], 0-50	2 [0-7], 0-50
CPAP (days)	14 [7-25], 0-47, n=118	15 [7-25], 0-47	14.5 [6.5-24], 0-47	15 [7-22], 1-47
Oxygen therapy (hours)	37 [2-210], 0-1515, n=105	12 [1-125], 0-1515, n=69	12 [2-82], 0-1515, n=45	33.5 [2-127], 0-1264, n=56
36week PMA oxygen requirement	34 (29%)	23 (28%)	8 (17%)	16 (25%)
Early MRI				
PMA at MRI (weeks.days)	31.6 [31.1-33.4], 29.3-35.2	32 [31.1-33.4], 29.3-35.2	32.3 [31.2-33.2], 30.2-35	
Weight at MRI (g)	1474 [1242-1684], 858-2715	1445 [1242-1670], 883-2715	1545 [1308-1729], 1023-2715	
Term MRI	n=105	n=77		
PMA at MRI (weeks.days)	40.4 [40-41.3], 38.3-46.4	40.6 [40-41.3], 38.3-46.4		40.5 [40-41.3], 38.3-42.5
Weight at MRI (g)	3000 [2700-3400], 1900-5150	3000 [2711-3500], 1900-5150		3000 [2700-3375], 1900-4114

Key: Data are presented as number (%), Median [IQR] or Mean (SD), range. Birth and maternal data was compared using unpaired t-tests for normally distributed continuous data, mann-whitney tests for non-normally distributed continuous data and chi2 tests for dichotomous variables. * Represents statistically significant differences between the included sample and the remaining infants which make up the 146 overall recruited cohort ($p<0.05$).

Supplementary Table 2: Comparison of outcome data for each sample included in analyses in this thesis, including detail of the participants excluded from diffusion MRI analyses due to failure of the automatic segmentation process.

	Early MRI & clinical correlates (paper 4; ch. 5)	**sMRI validation sample (paper 3; ch. 4)**	**Included in Early DWI analysis (ch. 6)**	**Excluded from Early DWI analyses due to failure of AS**	**Included in Term DWI analysis** (ch. 6)	**Excluded from Term DWI analyses due to failure of AS**
12 month outcomes	104	83	48	7 (5/7 with 12 month outcomes)	65	11 (10/11 with 12 month outcomes)
Age at assessment	12mo 4days (1wk 2days)	12mo 2days (1wk 2days)	12mo 4days (1wk 2days)	12mo 4days (1wk 6days)	12mo 3days (1wk 2days)	12mo 2days (1wk 2days)
Bayley III Motor	n=103			n=4		
Composite	97 [88-110], 46-127	97 [88-110], 46-127	100 [92.5-110], 67-127	95.5 [86.5-103], 85-103	97 [88-110], 52-127	98.5 [85-103], 46-121
Composite ≤100	66 (64%)	53 (64%)	30 (63%)	2 (50%)	42 (65%)	7 (70%)
Composite ≤85	16 (16%)	15 (18%)	5 (10%)	1 (25%)	8 (12%)	3 (30%)
Bayley III cognitive				n=4		
Composite	105 [100-110], 60-130	105 [100-115], 60-130	110 [100-115], 85-125	97.5 [90-105], 85-110	105 [100-110], 60-125	95 [85-110], 70-120
Composite ≤100	46 (45%)	36 (43%)	16 (33%)	3 (75%)	30 (46%)	6 (60%)
Composite ≤85	7 (7%)	6 (7%)	1 (2%)	1 (25%)	4 (6%)	3 (30%)
NSMDA	n=103			n=4		
total	184 [174-190], 98-199	185 [174-191], 98-199	186 [178.5-191], 146-199	184.5 [172-191.5], 164-194	185 [174-190], 117-199	183.5 [164-192], 98-194
AIMS	n=104			n=5		n=10
total	53 [50.5-54], 14-58	53 [50-54], 14-58	53 [52-53.5], 31-58	52 [47-57], 47-58	53 [51-53], 16-58	51 [47-56], 14-58
Total <5th percentile	11 (11%)	10 (12%)	1 (2%)	0	6 (9%)	2
Neurological Outcome	n=100	n=82	n=46	n=5	n=61	
Normal	70 (70%)	57 (70%)	32 (70%)	2 (40%)	45 (74%)	6 (60%)
Unspecified signs	25 (25%)	21 (26%)	12 (26%)	3 (60%)	16 (26%)	2 (20%)
Abnormal/likely CP	5 (5%)	4 (5%)	2 (4%)	0	0	2 (20%)
Confirmed CP	4 (4%)	3 (4%)	0	1 (20%)	1 (2%)	3 (30%)

Key: Data are presented as number (%), Median [IQR] or Mean (SD), range. AIMS, Alberta Infant Motor Scale; AS automatic segmentation; Bayley III, Bayley Scales of Infant and Toddler Development 3rd edition; CP, cerebral palsy; NSMDA, Neurosensory Motor Developmental Assessment; DWI diffusion weighted imaging.

Comparison of birth and maternal data across samples

The 27 infants recruited but who did not undergo Early MRI (and therefore did not progress further in the study) compared to the 119 who did undergo Early MRI, had a significantly lower birthweight, younger GA at birth, smaller head circumference, were more likely to be from a multiple birth and/or delivered by caesarean, and born to a family classified at higher social risk. This makes sense as 30% of the 27 infants did not undergo Early MRI because they were medically unstable, and 1 of the infants died. When comparing the 83 infants in the validation paper sample and the remaining 63 which make up the 146 recruited cohort, no statistically significant differences were found.

The 48 infants with useable Early MRI diffusion data compared to the 98 that make up the difference to 146: were born at older GA, with a greater birthweight, a lower incidence of chorioamnionitis and lower rates of social risk. All four of these variables have been associated with neurodevelopmental outcomes and so these differences are likely clinically relevant, and support the conclusion that the sample in the Early diffusion MRI group represent a lower risk sample than the overall cohort and other representative populations of very preterm infants. The 65 infants included in the Term MRI diffusion analyses only differed from the remaining 81 recruited by being born by caesarean section and had the lowest rate of higher social risk of all the samples.

When we examined the absolute values between the overall group of 146 and each of the other samples, the differences are not of a major magnitude and do not represent clinically important differences between the overall groups, except the lower incidence of chorioamnionitis, greater GA at birth and greater birthweight in the Early MRI diffusion group. For example, a difference of 2mm in head circumference between the 146 recruited and the 119 with Early MRI is less than the measurement error within the tool, and is likely of limited importance.

The presence of medical factors such as IVH, PVL, NEC, and sepsis were very similar across the groups, except for the absence of cases with hydrocephalus in the diffusion MRI analysis groups. The Early MRI diffusion analysis group also had a lower percentage of infants who had postnatal corticosteroids and a lower percentage of infants who were diagnosed with bronchopulmonary dysplasia (defined as an oxygen requirement at 36 weeks PMA).

Comparison of outcome data across samples

Table 2 presents detail of outcomes for each sample analysed, as well as specific data of the participants excluded from analyses in chapter 6 due to failure of the automatic segmentation process. The 104 infants in the first column represent all infants who had early MRI and 12 month outcomes. The samples in paper 3 and 4, as well as the Term MRI diffusion analysis sample in chapter 6 demonstrate similar motor and cognitive outcomes.

The infants included in the Early MRI diffusion analysis had a median 5 points higher cognitive composite score and 3 points higher motor composite score compared to the other groups. While this is not a very large difference, of likely importance is that for the AIMS, NSMDA, and Bayley III motor and cognitive composite scores, the lower limits were much higher in the Early diffusion sample. This indicates that none of the infants with the very poorest motor and cognitive outcomes were included in this latter sample. This is further evidenced by the much lower percentage of infants in the Early MRI diffusion sample who were classified as $<5^{th}$ percentile on the AIMS (2%), compared to 11% in the overall cohort and 12 % in the validation paper cohort. A similar pattern is seen with the cognitive outcome, with only 2% of the Early MRI diffusion analysis group having cognitive composite scores <85 compared with 7% of the other samples having cognitive composite scores <85, and 35% with cognitive composite scores <100 compared with 52% <100 in the overall cohort.

The prevalence of CP in the present cohort study was 4% (4/100). Although the study aimed to evaluate prediction of CP it was acknowledged that 12 months is early to confirm a diagnosis of CP. Most infants in the present cohort study have subsequently consented to a further study which follows the infants up at 2 years CA (Neurodevelopment of the preterm infant; NHMRC grant number 1084032). Two infants in the PPREMO study who were classified with ‘definitely abnormal neurological function /possible CP’ at 12 months CA were neurologically normal at subsequent clinical assessments at a local hospital and on their 2 year neurological assessment. One infant classified at 12 months CA with ‘unspecified signs’ on their neurological assessment was subsequently diagnosed at 2 years CA with hypotonic CP. That infant was born to parents who are both significantly intellectually impaired, and further investigations into genetic determinants for the child’s clinical presentation are in progress. Based on this information, the current total confirmed cases of CP in the PPREMO cohort is 4 out of 100 (4%). Due to the discrepancies between 12 month neurological findings, and confirmed outcomes at 2 years CA, the data collected from the paediatricians neurological assessment has been handled with caution in this thesis, and at times, only relationships with the validated motor assessments were presented, such as in paper 3.

The rate of CP of 4% is comparable with that in other contemporary cohorts. Data on rates of CP in the Australian state of Victoria for infants born between 2001 and 2009, report a rate of 3% for infants born between 28 and 31 weeks GA, and a rate of 8% in infants born <28 weeks GA[21]. A cohort of infants born <30 weeks GA in 2005-2006 in Melbourne, Australia reported a rate of CP of 6% (5/86). Data from 20 population based CP registers of infants born in 2003 reported prevalence rates of CP in very low birthweight infants (1000-1499g) of 3.6% and of extremely low birthweight infants (<1000g) of 4.2%[22]. In an Italian study of infants born in 2005-2006 with a birthweight of <1500g, 3.2% (5/156) were diagnosed with CP. These data indicate that in terms of CP outcome, our cohort is representative of contemporary populations of very preterm born infants.

If we examine our motor outcome data in relation to other studies of MRI in preterm infants, our Bayley III mean of 97 and SD of 14 is similar to a study of 45 infants who reported a mean 98 (SD 14)[28], and slightly higher than another Early MRI study of 52 infants who had a mean 93 (SD 14)[29]. A study of 65 infants that reported a lower mean of 85 (SD 11), had a mean GA at birth of their cohort of 26.6 and mean birthweight of 941g, which were both lower than the present study and likely explain the lower mean Bayley III motor score[30]. From this data we conclude that outcomes on the Bayley III motor composite score are comparable to other similar studies of very preterm infants with Early MRI. Interestingly, an Australian study with data of a term born reference sample of 211 infants, assessed at 12 months with the Bayley III, report a mean motor composite score of 97 (SD 12)[31, 32]. This indicates that our preterm cohort performed comparably to healthy term born infants when assessed with the Bayley III.

A study of 86 infants born <30 weeks GA between 2005 and 2006, also assessed motor outcome at 12 months CA with the AIMS and NSMDA[33]. Their cohort's mean GA at birth was a week younger than the present study, but their rates of IVH grade III and IV, PVL and oxygen requirement at 36 weeks was the same as the present cohort, and their rate of CP was 6%[33]. Interestingly, although the rates of CP were similar, 35% of their cohort compared with our 11%, were classified as <5th percentile on the AIMS, and 18% compared with our 8%, were classified as having mild-severe dysfunction on the NSMDA.

In conclusion, the data presented here confirm that the sample included in the Early MRI diffusion analyses in this chapter represent a lower risk cohort with more favourable birth characteristics and better motor and cognitive outcomes compared to the overall study sample. None of the infants with confirmed CP were included in the Early MRI diffusion analysis sample. Infants excluded due to

failure of automatic segmentation processes had poorer motor and cognitive outcomes than those included in analyses with diffusion data.

Brain macrostructure and microstructure have now been examined, together with clinical correlates and the relationships with 12 month motor outcomes which completes all stated aims of this thesis. I now turn to the general discussion and conclusions in chapter 7 to be drawn from these series of studies.

Chapter 7: Discussion and Conclusions

7.1 Summary of Research Results

This chapter presents a summary of findings for each aim. This is followed by a general discussion synthesizing and critiquing the findings in the context of other published literature on Early MRI and the relationship to outcomes in very preterm populations. Study strengths and limitations are then elucidated, followed by clinical and research implications and recommendations for future research.

Aim 1 *To examine the diagnostic accuracy of early MRI (<36 weeks PMA) to determine adverse motor outcomes or a confirmed diagnosis of CP, at or beyond one year CA in infants born preterm.*

The systematic review evaluated 30 papers, 5 of which reported diagnostic accuracy and another 5 reported data sufficient for diagnostic accuracy to be calculated. Meta-analyses revealed that Early structural MRI global scores had good sensitivity and specificity to detect a later diagnosis of CP. To determine motor outcomes, global scores had a higher sensitivity and specificity than WM scores, but both were lower than for detection of CP. The lack of reporting of diagnostic accuracy in the majority of studies limited the ability to interpret the findings of Early MRI and their potential to provide prognostic information for use in routine clinical care.

This systematic review is current at the time of submission of this thesis and evaluates all published literature until March 2017. Systematic searching of the literature at the start of this PhD (2012) revealed only 11 papers of Early MRI and motor outcomes, which included only 1 study of 12 participants with Early diffusion MRI and motor/CP outcomes[20]. The need for further investigation of both structural and advanced diffusion MRI Early in the neonatal period for infants born very preterm was clearly warranted. I therefore set out to acquire both structural and advanced diffusion imaging coupled with concurrent clinical measures and neurodevelopmental follow up to 12 months CA in a large, prospectively collected cohort of very preterm infants.

Initial investigations of the structural MRI data were required before progressing to evaluation and interpretation of the advanced diffusion data. The need for a comprehensive Early structural MRI scoring system that incorporated evaluation of deep GM and the cerebellum was identified, as well as the requirement to identify and report not only associations between Early MRI findings and motor outcomes, but also measures of diagnostic accuracy to facilitate clinical utility.

Aim 2 *To validate a structural MRI scoring system previously developed for very preterm infants at TEA in a cohort of infants born <31 weeks PMA with MRI between 29 and 35 weeks PMA. The study aimed to establish predictive validity for motor and cognitive outcomes at 12 months CA. Secondary aims were to examine inter- and intrarater reproducibility and to examine relationships between global brain abnormality categories and known perinatal risk factors.*

Early MRI scores were associated with both motor and cognitive outcomes at 12 months CA in the cohort of infants born <31 weeks PMA, validating the scoring system and addressing Aim 2 of this thesis. Early MRI WM, deep GM and global brain abnormality scores were associated with Bayley III motor and cognitive scores and outcome on the NSMDA. Early cerebellar scores were also associated with outcome on the NSMDA. These associations were reconfirmed at Term MRI. Cerebellar scores at Term MRI were also associated with Bayley III motor and cognitive outcomes at 12 months CA. Inter- and intrarater reproducibility of the scoring system was demonstrated. Reliability and agreement were excellent for the Early and Term MRI global score and all subscales except cortical GM. Early MRI scores detected both motor and cognitive outcomes with good specificity and lower sensitivity.

Inclusion of evaluation of deep GM and cerebellar structures and the use of regional measures to capture the impact of secondary growth impairment differentiates this scale from previously validated methods for Early MRI[10, 11]. Of the subscales and overall global score, deep GM scores demonstrated the strongest associations with motor and cognitive outcomes and the highest sensitivity and specificity for motor outcomes. These findings support inclusion of evaluation of deep GM in Early MRI scoring systems. Early MRI cerebellar scores were associated with the NSMDA but not Bayley III motor outcome. This is an interesting finding and might be related to the Bayley III assessing motor achievement while the NSMDA evaluates motor quality, including postural and balance reactions, functions known to be modulated by the cerebellum.

The method to correct for PMA at MRI for regional measurements developed in this study effectively ‘age standardises’ the data. This ensured any differences found in regional measures between infants were not an effect of head or brain size due to PMA at MRI, but rather differences due to growth impairment. The use of term reference data to generate cut-points for scoring of the regional measurements was a potentially valuable modification to the original scale upon which this scale was based[26]. Having addressed Aim 2, the next aim was to elucidate associations between these Early and Term MRI scores and concurrent clinical measures of motor, neurological and neurobehavioural function.

Aim 3 *To examine the structure-function relationships between structural MRI brain abnormality scores and concurrent clinical measures of neuromotor, neurological and neurobehavioral performance at 30-32 weeks PMA (Early MRI) and again at 40-42 weeks PMA (Term MRI). A secondary aim was to evaluate which clinical measures demonstrated the strongest association with a) Early MRI and b) Term MRI.*

This study aimed to define the associations between clinical measures and concurrently collected Early and Term structural MRI scores. A broad range of clinical tools were tested evaluating neurological, motor and neurobehavioural function. Both Early and Term MRI scores were found to demonstrate associations with concurrent clinical measures. Cerebellar subscale scores demonstrated the strongest associations with clinical measures at Early MRI, and predominantly with neurological items of the tests. Early MRI cerebellar scores were associated with the domain of reflexes on the HNNE, hypertonicity on the NNNS, the Premie-Neuro neurological subscale and cramped-synchronised GMs. At Term MRI, the strongest associations were with the TIMP, a neuromotor test. The TIMP demonstrated associations with WM, deep GM and global MRI scores indicating that increasing severity of brain injury was associated with poorer motor function.

An interesting finding was the lack of associations between Early WM scores and clinical measures. In contrast, the majority of Term MRI studies have found WM abnormalities to correlate with concurrent clinical performance, and our Term MRI data confirmed this with strong associations found with motor performance on the TIMP as well as abnormal signs on the HNNE and hypertonicity on the NNNS. These findings further support evaluation of deep GM structures and the cerebellum in Early MRI studies of preterm infants.

To our knowledge, this work presents the first structure-function relationships between Early structural MRI and concurrent clinical measures of motor, neurological and neurobehavioral function in infants born very preterm. It is also the first study of Early and Term clinical associations with a structural MRI scoring system that includes evaluation of deep GM and the cerebellum. It addresses and achieves the third aim of the thesis, and the next step was to begin evaluation of the diffusion MRI data.

Aim 4 *To evaluate brain microstructure on Early and Term MRI in regions known to be involved in motor function, the corpus callosum, posterior limb of the internal capsule, and cerebral peduncle and then examine the association of these early microstructural measures with motor outcome at 12*

months CA. A secondary aim was to compare preterm brain microstructure in the 3 defined regions at Term MRI with a term born reference sample imaged at approximately 40-42 weeks PMA.

The systematic review in chapter 2 identified studies which found lower FA in the PLIC and corpus callosum to be associated with poorer motor outcomes[20, 34, 35]. This informed the selection of regions for inclusion in these initial analyses of the diffusion MRI data. In this cohort of infants born <31 weeks GA with useable diffusion imaging and 12 month outcome data available, no associations were found between FA or MD at Early MRI in the corpus callosum, PLIC and cerebral peduncles and motor or neurological outcomes. Term MRI FA was also not associated with motor or neurological outcomes at 12 months CA. Left corpus callosum MD at Term MRI was associated with neurological outcome; right cerebral peduncle MD at Term MRI was associated with motor outcome on the AIMS and NSMDA. When the preterm cohort Term MRI data was compared to the term reference group, corpus callosum and global FA and MD were significantly different between the groups.

Exclusion of infants with diffusion data of useable quality but who failed the automatic segmentation process due to the presence of structural lesions distorting the brain tissue, likely impacted our results as those with the worst brain structure were excluded. All infants in the cohort who developed CP were excluded from the analyses of Early MRI with 12 month outcomes. The results presented in chapter 6 address Aim 4.

7.2 General discussion

Identifying very preterm infants at risk of adverse motor outcomes and CP using Early MRI and clinical correlates was the premise underpinning this thesis. The results confirm that prediction of motor outcomes is possible earlier than the current standard of TEA. The scoring system developed for Early structural MRI was valid and reproducible and the data are consistent with previous studies of early structural MRI which show significant associations with motor and neurodevelopmental outcomes [10, 11]. The Early structural MRI scoring system contributes to the current literature in 3 important ways. It is the first scoring system for Early structural MRI that includes evaluation of deep GM and the cerebellum, and includes regional measurements of the brain which aim to quantify the secondary impacts on brain growth and development following structural brain injury. Clinical correlates of the Early MRI structural scores are presented, and to our knowledge are the only concurrent clinical associations reported for Early structural MRI. Analysis of the Early diffusion MRI demonstrated that microstructural development measured by

fractional anisotropy and mean diffusivity in the corpus callosum, PLIC and cerebral peduncle, were not associated with 12 month motor or neurological outcomes.

The process of validating the Early structural MRI scoring system examined the associations between Early MRI scores and 12 month motor outcomes and then the sensitivity and specificity of Early MRI scores to determine motor and cognitive outcomes. Establishing diagnostic accuracy is more challenging than finding associations, a fact demonstrated in the validation study where strong associations were found, but the sensitivity and specificity of the scores to predict motor and cognitive outcomes were more limited. The preterm infant brain is developing rapidly following birth and up to TEA, there are a myriad of potential perinatal, environmental and genetic factors that play a role in the developmental trajectory. Brain MRI, evaluated macro- or microstructurally may not be sufficiently sensitive to capture some or all of the brain changes related to premature birth and the medical and social sequelae.

There is now clear evidence that the rate of CP is declining globally in infants born preterm[21, 22]. This makes the task of predicting an outcome of CP following premature birth even more difficult, and requires larger samples of preterm born infants in prospective cohort studies. Adverse motor outcomes in very preterm born infants have also been reported as lower in contemporary cohorts compared with cohorts from the decade prior[36]. Prevalence of CP or adverse motor outcomes in a cohort has a direct impact on positive and negative predictive value; a lower prevalence results in poorer predictive values for any diagnostic test[24, 37]. Studies of diagnostic accuracy in preterm populations frequently employ sensitivity and specificity measures which impart valuable diagnostic information, as well as enable data from multiple studies with different prevalence rates of CP to be combined in meta-analyses. Sensitivity and specificity are not measures of prediction: at a defined sensitivity and specificity, where there is a low prevalence of the outcome of interest (CP or adverse motor outcomes), it becomes more difficult to predict from an adverse finding on MRI or clinical tests, and easier to predict from a normal MRI or clinical test result[24, 37, 38]. As discussed in detail in chapter 6, the rate of CP in the PPREMO cohort of 4% is consistent with other contemporaneous cohorts of very preterm born infants.

Research in representative samples of very preterm born infants share a fundamental difficulty; class imbalance in the data[39]. Although very preterm infants display poorer motor outcomes than their term born peers, within a representative cohort of very preterm infants there are commonly only a very small number of infants who have significantly worse motor outcomes than the rest of the cohort. It is often the small number of infants with CP who display these significantly lower

motor scores. This results in the data being heavily skewed towards relatively better motor outcomes, and is referred to as class imbalance, where a sample contains only a few abnormal cases. When regression analyses are conducted to identify relationships between MRI scores and later outcomes, the few cases with markedly worse motor outcomes effectively drive the associations. Often, if the few severe cases are removed, the relationships fail to remain significant. Complex statistical methods have been developed to address class imbalance, such as LSI (local synthetic instances) or SMOTE (synthetic minority over sampling technique), but the complexity of these methods preclude them from widespread use[40, 41]. Class imbalance in the data will remain a core challenge for research in populations of preterm infants, especially given a declining rate of CP in this population.

The sensitivity and specificity of the structural MRI scoring system developed in this thesis share similarities with studies of TEA MRI in preterm infants[6, 25, 33]. All scoring systems had higher specificity than sensitivity for motor and cognitive outcomes indicating that a normal MRI is highly indicative of a normal outcome, whereas infants with moderate to severe abnormalities on MRI progress to variable motor/cognitive outcomes. The global score from the Early structural MRI scoring system developed in this thesis had a 50% sensitivity and 86% specificity for cognitive outcomes on the Bayley III at 12 months CA[42], higher than the widely used qualitative scoring system for structural MRI at TEA[6, 9]which reported a sensitivity 41% and specificity 84% for cognitive outcomes at 2 years CA. For motor outcomes, our Early MRI global score determined motor outcome on the NSMDA with a sensitivity of 43% and specificity of 86%, comparable with sensitivity 44% and specificity 96% from a study of TEA WM abnormality to determine motor outcome on the NSMDA[33]. Most subscales and the overall global total in our structural MRI scoring system, demonstrated higher sensitivity at Early MRI compared to Term MRI, although the specificities were comparable at both time points[42].

Our studies of Early structural MRI scores and 12 month outcomes (Chapter 4), and concurrent clinical associations with Early structural MRI scores (Chapter 5), present important findings in relation to the cerebellum. The cerebellum grows rapidly in the third trimester, increasing in size by 258% between 30 and 40 weeks PMA whilst growing at a faster rate than any other cerebral structure[43, 44]. This makes the cerebellum particularly vulnerable to perturbations and injury, both in terms of injury caused by infarction, infection or haemorrhage, or due to impaired or restricted development[45]. Cerebellar abnormalities have been associated with adverse cognitive, language and behavioural abnormalities in infants born preterm[46], and a reduction in remote cerebral cortical growth[47]. Early and Term MRI cerebellar scores in the present cohort were associated with

cognitive outcome on the Bayley III and motor outcome on the NSMDA. Early MRI cerebellar scores also demonstrated the strongest associations of the MRI subscales and global score, with concurrent clinical measures, notably with neurological elements of the various clinical measures. These findings support inclusion of evaluation of the cerebellum in studies of Early MRI.

Generalisability of findings from this PPREMO study has been discussed in the summary of chapter 6. The recruited sample is representative of contemporary preterm populations born <31 weeks GA, with comparable outcomes in terms of rates of CP. During development of the study protocol, the literature was comprehensively reviewed to determine which perinatal variables were reported as risk factors for poorer motor development or an outcome of CP. The most relevant risk factors were collected in the cohort study and have been presented in Table 1 (Summary of Chapter 6). Examination of these perinatal data reveals that most perinatal and clinical features of our cohort are similar to contemporary cohorts, except that we seemed to have a significantly lower rate of sepsis. Only 4% of our cohort had culture positive sepsis, compared with approximately 30%[29, 30, 48] in other cohorts with Early MRI. Confirmed sepsis increases the risk of adverse motor and cognitive outcomes and CP[5, 49]. The low rate of nosocomial infection in our study would have resulted in less inflammatory triggers for brain inflammation which may explain the better motor outcomes relative to some other studies[30, 33].

Selection of perinatal risk factors to correct for in statistical analyses is challenging. A number of perinatal variables demonstrate co-linearity (e.g. GA at birth and birth weight), and addition of perinatal factors may even improve certain models. For example, a recent study reported diffusion MRI network analysis predicted motor and cognitive outcomes when presented as a combined model of network measure features and perinatal data including sex, GA at birth and WMI [40]. When the analysis was replicated in the same cohort but without the addition of the perinatal risk factors, network analysis alone failed to predict motor and cognitive outcomes[41].

In the validation paper, the relationship between the perinatal risk factors, and increasing severity of MRI abnormality scores was evaluated. Gestational age at birth, birthweight, retinopathy of prematurity and days of ventilation were significantly positively associated with MRI scores at both Early and Term MRI. In addition, Early MRI abnormality scores were associated with the presence of patent ductus arteriosus, administration of postnatal corticosteroids, and oxygen therapy. Term MRI abnormality scores were associated with higher social risk, an oxygen requirement at 36 weeks PMA and a requirement for home oxygen. In relation to the Early MRI abnormality scores, these associations are perhaps not surprising as they suggest the babies most acutely sick soon after birth

and likely to go on to have the worst lung outcomes already had experienced the stressors resulting in acute changes in the structural MRI. To a certain extent the same factors held over to the Term MRI, but of interest is that fact that social risk now had an association with adverse MRI score suggesting that postnatal, potentially cumulative events had operated in the intervening period to leave an MRI biomarker by the time of the Term MRI.

7.3 Strengths of the Thesis

This thesis describes the successful design, implementation and analysis of a complex and challenging study involving fragile preterm infants with 2 MRIs and multiple outcome measures concurrent with MRI, and at 3 and 12 months CA. A prospective cohort study design, ranked higher in the hierarchy of evidence than a retrospective study design, was coupled with strong methodological quality. Scorers of MRI were blinded to clinical history and concurrent clinical assessment findings. Clinical assessors at Early and Term time points were blinded to MRI findings and clinical history. Outcome assessors were blinded to all early MRI and clinical findings. Clinical assessors were trained and accredited in all relevant assessment measures, and raters of GMs videos were advanced, accredited raters.

All infants were imaged on the same MRI scanner with the same MR protocol and at high field strength (3T). Advanced diffusion sequences were acquired in addition to structural images. Inclusion of concurrent clinical correlates for MRI differentiates this study from the majority of other Early MRI studies. Validated tools were utilised for outcome assessments. The Bayley III is the outcome of choice for most preterm follow up studies, however it has limited predictive validity for later motor and cognitive outcomes, and concerns have been raised that it underestimates motor and cognitive delays. The current study protocol included assessment with the AIMS and NSMDA at 12 months CA to supplement motor outcome data obtained using the Bayley III.

Recruitment of a large, unselected consecutive cohort, representative of contemporaneous cohorts of preterm infants born <31 weeks ensures good generalisability of the results. Very good retention of study participants to 12 months CA (87%) was achieved. Systematic literature searching at the start ensured relevant study design and facilitated implementation of the study. The systematic review presented in this thesis evaluated the literature to March 2017 and therefore presents an up to date, detailed evaluation of the literature regarding Early MRI and motor outcomes in infants born preterm, including meta-analyses.

7.4 Limitations of the Thesis

A number of potential limitations can be identified. Twelve months CA is early to determine motor and cognitive outcomes for very preterm infants. Follow up at 2 years CA would have been preferable, however that was not possible within the timeframe available for this thesis. The research team that includes me has been successful in procuring funding for a follow on project that will enable follow up at 2 years CA of the PPREMO participants.

It was acknowledged from the outset that 12 months CA would be early to evaluate the presence of, or confidently determine an absence of CP. I observed in some of the earlier PPREMO recruits that some who presented with abnormal neurological function at 12 months CA progressed to normal motor and neurological outcomes when assessed at 2 years CA in the follow on study. This limits the confidence with which we are able to use our 12 month neurological assessment data. Use of another standardised neurological assessment such as the Hammersmith Infant Neurological Examination (HINE), which generates a continuous score for neurological outcome, may have complemented our current neurological assessment[50-52].

The atlas-based automatic segmentation method utilised with the diffusion MRI data resulted in exclusion of infants with significant structural brain lesions. Further automated approaches or manual segmentation will be needed if future research questions require analyses to include the full representative cohort. Infants who did not have a successful Early MRI did not progress through the study. The study design should possibly have allowed follow up of all infants recruited, to be able to determine if those who were recruited but had no MRI were similar or different in outcome to the samples included in each of the other analyses. Data of acquired medical factors in Table 1, chapter 6 was not collected for infants recruited but with no Early MRI, and limited comparison of these data for the full recruited sample compared to each of the analysed samples. This is something that could be addressed retrospectively and would enhance comparisons between groups for future work in this cohort.

A small amount of attrition occurred within this study, 87% of infants with Early MRI were followed up at 12 months CA. This follow rate was better than that of many other studies of early MRI in which rates ranged from 30-75%[11, 20, 28, 53-58]. Potential bias is created by incomplete follow-up. Poorer outcomes are reported for preterm infants who fail to return for follow up compared to those who are followed up[59], and greater biological and social risk factors for disabilities occur in those who fail to access follow up services[60].

7.5 Clinical and Research Implications and Recommendations for Future Research

The findings of this research have a number of important clinical and research implications. Early MRI offers the benefit of earlier identification of brain injury and/or impaired development with potential for an earlier start of interventions. In the absence of interventions, early identification has the potential to increase anxiety for parents at a vulnerable and immensely difficult time[61]. The landscape is changing however, with intervention studies becoming available which aim to improve infant and family outcomes[62-64]. Recent 8 year follow up data from the Vibes + study showed that mothers of babies who received intervention with psychological support in the first year after preterm birth demonstrated sustained improvements in mental health to 8 years[65]. While the specific early intervention applied in that study demonstrated limited effects on motor and cognitive development in the preterm born children, sustained improvements in parental mental health represent a vitally important contribution[66]. Research into neuroprotective strategies such as hypothermia, magnesium sulphate, erythropoietin, creatine, and melatonin are all underway, as well as early massage, developmental care and environmental enrichment and will require methods to identify infants for inclusion in studies, as well as biomarkers to evaluate outcomes.

The GMs assessment has higher sensitivity and specificity for an outcome of CP in preterm populations than MRI, and should be included in clinical and research settings of very preterm infants[67]. International recommendations accepted for publication in the journal JAMA Pediatrics recommend both MRI and GMs assessment for early detection of CP and to diagnose others 'at high risk of CP' to enable early interventions to be implemented (personal communication). General Movement assessment data was collected and future work is planned to evaluate the predictive validity of GMs along with the other early clinical data collected. No studies to date have evaluated a combination of Early MRI and clinical measures to determine motor outcomes or CP. Studies of Term MRI have demonstrated improved prediction of outcomes, when MRI data and neuromotor or neurological data are combined[33, 67-70]. Evaluation of the combination of Early MRI and concurrent clinical measures to predict motor outcomes and CP is planned with the data collected in the PPREMO study.

Longitudinal changes in diffusion measures between Early and Term MRI have been related to motor outcomes[20, 30, 34]. A slower increase in FA between Early and Term MRI in the PLIC and occipital WM was reported as associated with poorer motor outcomes in one study[20] but not in another[34]. A slower increase in FA in the basal nuclei[34], and a difference in the slope of FA between left & right inferior temporal lobe, where FA increases more slowly on the left than the right[30] have been reported as associated with poorer motor outcomes. Structural and diffusion imaging measures

of growth of the hippocampus between Early and Term MRI were not associated with motor outcomes[71]. Growth of the putamen and caudate predicted cognitive outcomes[72]. We have examined the relationships between MRI measures at each time point and later motor outcomes as presented in Chapters 4 and 6 of this thesis. Future work could examine growth and/or maturation of structures between Early and Term MRI and the ability of these changes (rate/magnitude of change) to determine outcomes.

This thesis focussed on motor outcomes and CP. Future work will examine the structural and diffusion imaging findings in relation to cognitive and behavioural outcomes. Further structural and microstructural evaluation of the cerebellum is indicated given the findings in this study of relationships between Early structural MRI cerebellar scores and 12 month motor and cognitive outcomes, as well as strong associations with concurrent clinical measures.

Evidence of injury to some structures appears over time[73] as well as growth impairment becoming more evident at Term MRI compared to Early MRI as demonstrated in paper 3 of this thesis[42]. An example of this is the thalamus, where no volumetric differences were noted on Early MRI but significant differences were evident on Term MRI, suggesting that damage to the thalamus is secondary to brain injury in the corticospinal tract occurring earlier[73]. This suggests that when looking for biomarkers at Early MRI, future research potentially needs to examine different structures to those known at TEA to be related to outcomes. A study of spectroscopic imaging in normative preterm infants found the corticospinal tract to have the highest NAA/Cho ratio suggesting that of the 14 regions of interest that they examined, the CST appeared to have matured first[74]. This may support choosing the corticospinal tract as a structure to examine at Early MRI. That report also found that the parietal white matter had higher NAA/Cho ratio than the frontal white matter, possibly demonstrating the sensory pathway is quicker to mature compared with the motor pathway.

The structural MRI scoring system developed and described in this thesis had good specificity for motor outcomes but relatively low sensitivity. Future research of this and other MRI scoring systems could explore ways to improve the diagnostic accuracy of the existing scoring systems. Removal of redundant scoring items and optimisation of cut points for MRI scores and for outcomes could be evaluated. Weighting individual items or subscale scores within the global total score may improve diagnostic accuracy. Data augmentation methods such as local synthetic instances (LSI[40]) and synthetic minority over-sampling technique (SMOTE[41, 75]) to address class

imbalance issues inherent in studies of preterm infants, and/or machine learning or deep learning statistical modelling could be employed.

Future research should continue to search for new, very early biomarkers of outcome for very preterm born infants. Early and advanced imaging potentially has a lot to offer in this task. Acquisition types include structural and diffusion as presented in this thesis, as well as spectroscopic, functional and quantitative T1 and T2 methods. In addition to qualitative scoring of structural images and region of interest analyses in diffusion MRI, cortical thickness measures, tractography including along-tract analysis[76], voxel based analyses such as tract based spatial statistics[77], fixel-based analysis[78], connectome[79] and network analyses[80] potentially offer valuable insights into early brain development. High angular resolution diffusion imaging acquires more directions and has a higher diffusion weighting than standard diffusion tensor imaging, addressing the challenges of crossing fibres and can be used for constrained spherical deconvolution[81] or q-ball imaging[82]. Multi-shell approaches with multiple diffusion weightings or different number of directions per diffusion weighting can be used with NODDI[83], diffusion kurtosis imaging[84] or multi-shell multi-tissue constrained spherical deconvolution[85]. Additional microstructural measures available from NODDI include, intra-cellular volume fraction, isotropic volume fraction and orientation dispersion[83, 86, 87]. From FOD, fibre density, fibre bundle cross-section and combined measures of fibre density and bundle cross-section can be calculated[78]. From diffusion kurtosis imaging: intra-axonal water fraction, intra-axonal axial diffusivity, extra-axonal axial and radial diffusivities can be calculated[84, 86, 88].

7.6 Conclusion

In this thesis I have successfully investigated “The relationship between brain structure and function of very preterm infants, and the ability to predict neurodevelopmental outcomes”. I have generated a dataset of Early and Term MRI at high field strength (3T), with advanced diffusion MRI acquisition, concurrent clinical correlates and motor and cognitive outcomes to 12 months corrected age. I have validated a structural MRI scoring system for use at Early and Term MRI and assessed the relationship between qualitative scores of brain macrostructure and concurrent clinical function. I have investigated the relationship between early brain microstructure and motor and neurological outcome and, in the process, profiled and described the cohort. The challenge and opportunity from here will be to maximise and optimise the learning from this valuable dataset.

References

1. de Kieviet JF, Piek JP, Aarnoudse-Moens CS, Oosterlaan J. Motor development in very preterm and very low-birth-weight children from birth to adolescence: a meta-analysis. *JAMA* 2009; **302**: 2235-42.

2. Himpens E, Van den Broeck C, Oostra A, Calders P, Vanhaesebrouck P. Prevalence, type, distribution, and severity of cerebral palsy in relation to gestational age: a meta-analytic review. *Dev Med Child Neurol* 2008; **50**: 334-40.

3. Williams J, Lee KJ, Anderson PJ. Prevalence of motor-skill impairment in preterm children who do not develop cerebral palsy: a systematic review. *Dev Med Child Neurol* 2010; **52**: 232-7.

4. Aarnoudse-Moens CS, Weisglas-Kuperus N, van Goudoever JB, Oosterlaan J. Meta-analysis of neurobehavioral outcomes in very preterm and/or very low birth weight children. *Pediatrics* 2009; **124**: 717-28.

5. Synnes A, Luu TM, Moddemann D, et al. Determinants of developmental outcomes in a very preterm Canadian cohort. *Arch Dis Child Fetal Neonatal Ed* 2017; **102**: F1-9.

6. Woodward LJ, Anderson PJ, Austin NC, Howard K, Inder TE. Neonatal MRI to predict neurodevelopmental outcomes in preterm infants. *N Engl J Med* 2006; **355**: 685-94.

7. Spittle AJ, Cheong J, Doyle LW, et al. Neonatal white matter abnormality predicts childhood motor impairment in very preterm children. *Dev Med Child Neurol* 2011; **53**: 1000-6.

8. Spittle AJ, Cheong J, Doyle LW, et al. White matter abnormality at term is predictive of developmental co-ordination disorder at 5 years of age for children born very preterm. *J Paediatr Child Health* 2010; **46**: 15.

9. Inder TE, Wells SJ, Mogridge NB, Spencer C, Volpe JJ. Defining the nature of the cerebral abnormalities in the premature infant: a qualitative magnetic resonance imaging study. *J Pediatr* 2003; **143**: 171-9.

10. Miller SP, Ferriero DM, Leonard C, et al. Early brain injury in premature newborns detected with magnetic resonance imaging is associated with adverse early neurodevelopmental outcome. *J Pediatr* 2005; **147**: 609-16.

11. Sie LT, Hart AA, van Hof J, et al. Predictive value of neonatal MRI with respect to late MRI findings and clinical outcome. A study in infants with periventricular densities on neonatal ultrasound. *Neuropediatrics* 2005; **36**: 78-89.

12. Arzoumanian Y, Mirmiran M, Barnes PD, et al. Diffusion tensor brain imaging findings at term-equivalent age may predict neurologic abnormalities in low birth weight preterm infants. *AJNR Am J Neuroradiol* 2003; **24**: 1646-53.

13. De Bruine FT, Van Wezel-Meijler G, Leijser LM, et al. Tractography of white-matter tracts in very preterm infants: a 2-year follow-up study. *Dev Med Child Neurol* 2013; **55**: 427-33.

14. Rose J, Butler EE, Lamont LE, Barnes PD, Atlas SW, Stevenson DK. Neonatal brain structure on MRI and diffusion tensor imaging, sex, and neurodevelopment in very-low-birthweight preterm children. *Dev Med Child Neurol* 2009; **51**: 526-35.

15. Kaukola T, Perhomaa M, Vainionpaa L, et al. Apparent diffusion coefficient on magnetic resonance imaging in pons and in corona radiata and relation with the neurophysiologic measurement and the outcome in very preterm infants. *Neonatology* 2010; **97**: 15-21.

16. van Kooij BJ, de Vries LS, Ball G, et al. Neonatal tract-based spatial statistics findings and outcome in preterm infants. *AJNR Am J Neuroradiol* 2012; **33**: 188-94.

17. Thompson DK, Inder TE, Faggian N, et al. Corpus callosum alterations in very preterm infants: perinatal correlates and 2 year neurodevelopmental outcomes. *Neuroimage* 2012; **59**: 3571-81.

18. Thompson DK, Lee KJ, Egan GF, et al. Regional white matter microstructure in very preterm infants: predictors and 7 year outcomes. *Cortex* 2014; **52**: 60-74.

19. Brouwer MJ, van Kooij BJ, van Haastert IC, et al. Sequential cranial ultrasound and cerebellar diffusion weighted imaging contribute to the early prognosis of neurodevelopmental outcome in preterm infants. *PLoS One* 2014; **9**: e109556.

20. Drobyshevsky A, Bregman J, Storey P, et al. Serial diffusion tensor imaging detects white matter changes that correlate with motor outcome in premature infants. *Dev Neurosci* 2007; **29**: 289-301.

21. Reid SM, Meehan E, McIntyre S, et al. Temporal trends in cerebral palsy by impairment severity and birth gestation. *Dev Med Child Neurol* 2016; **58 Suppl 2**: 25-35.

22. Sellier E, Platt MJ, Andersen GL, et al. Decreasing prevalence in cerebral palsy: a multi-site European population-based study, 1980 to 2003. *Dev Med Child Neurol* 2016; **58**: 85-92.

23. Orcesi S, Olivieri I, Longo S, et al. Neurodevelopmental outcome of preterm very low birth weight infants born from 2005 to 2007. *Eur J Paediatr Neurol* 2012; **16**: 716-23.

24. Hanna SE. High and variable prevalence does matter in reviews of diagnostic accuracy in cerebral palsy. *Dev Med Child Neurol* 2013; **55**: 397-8.

25. Van't Hooft J, van der Lee JH, Opmeer BC, et al. Predicting developmental outcomes in premature infants by term equivalent MRI: systematic review and meta-analysis. *Syst Rev* 2015; **4**: 71.

26. Kidokoro H, Neil JJ, Inder TE. New MR imaging assessment tool to define brain abnormalities in very preterm infants at term. *AJNR Am J Neuroradiol* 2013; **34**: 2208-14.

27. Rose J, Vassar R, Cahill-Rowley K, Guzman XS, Stevenson DK, Barnea-Goraly N. Brain microstructural development at near-term age in very-low-birth-weight preterm infants: an atlas-based diffusion imaging study. *Neuroimage* 2014; **86**: 244-56.

28. Tam EW, Chau V, Barkovich AJ, et al. Early postnatal docosahexaenoic acid levels and improved preterm brain development. *Pediatr Res* 2016; **79**: 723-30.

29. Young JM, Morgan BR, Whyte HE, et al. Longitudinal Study of White Matter Development and Outcomes in Children Born Very Preterm. *Cereb Cortex* 2016: 1-12.

30. Rogers CE, Smyser T, Smyser CD, Shimony J, Inder TE, Neil JJ. Regional white matter development in very preterm infants: perinatal predictors and early developmental outcomes. *Pediatr Res* 2016; **79**: 87-95.

31. Walker K, Badawi N, Halliday R, Laing S. Brief report: Performance of Australian children at one year of age on the Bayley Scales of Infant and Toddler Development (Version III). *The Australian Educational and Developmental Psychologist* 2010; **27**: 54-8.

32. Walker K, Badawi N, Halliday R, et al. Early developmental outcomes following major noncardiac and cardiac surgery in term infants: a population-based study. *J Pediatr* 2012; **161**: 748-52 e1.

33. Spittle AJ, Boyd RN, Inder TE, Doyle LW. Predicting motor development in very preterm infants at 12 months' corrected age: the role of qualitative magnetic resonance imaging and general movements assessments. *Pediatrics* 2009; **123**: 512-7.

34. Chau V, Synnes A, Grunau RE, Poskitt KJ, Brant R, Miller SP. Abnormal brain maturation in preterm neonates associated with adverse developmental outcomes. *Neurology* 2013; **81**: 2082-9.

35. Malavolti AM, Chau V, Brown-Lum M, et al. Association between corpus callosum development on magnetic resonance imaging and diffusion tensor imaging, and neurodevelopmental outcome in neonates born very preterm. *Dev Med Child Neurol* 2017; **59**: 433-40.

36. Harris SL, Austin NC, Battin M, et al. In-hospital morbidity and brain metrics of preterm neonates born 1998-2009. *N Z Med J* 2016; **129**: 94-107.

37. Leeflang MM, Bossuyt PM, Irwig L. Diagnostic test accuracy may vary with prevalence: implications for evidence-based diagnosis. *J Clin Epidemiol* 2009; **62**: 5-12.

38. Simundic AM. Measures of Diagnostic Accuracy: Basic Definitions. *Ejifcc* 2009; **19**: 203-11.

39. Japkowicz N, Stephen S. The class imbalance problem: A systematic study. *Intelligent data analysis* 2002; **6**: 429-49.

40. Brown CJ, Miller SP, Booth BG, et al. Prediction of motor function in very preterm infants using connectome features and local synthetic instances. *International Conference on Medical Image Computing and Computer-Assisted Intervention* 2015: 69-76.

41. Kawahara J, Brown CJ, Miller SP, et al. BrainNetCNN: Convolutional neural networks for brain networks; towards predicting neurodevelopment. *Neuroimage* 2017; **146**: 1038-49.

42. George JM, Fiori S, Fripp J, et al. Validation of an MRI Brain Injury and Growth Scoring System in Very Preterm Infants Scanned at 29- to 35-Week Postmenstrual Age. *AJNR Am J Neuroradiol* 2017: May 18. doi: 0.3174/ajnr.A5191 [Epub ahead of print].

43. Kersbergen KJ, Makropoulos A, Aljabar P, et al. Longitudinal Regional Brain Development and Clinical Risk Factors in Extremely Preterm Infants. *J Pediatr* 2016; **178**: 93-100.e6.

44. Limperopoulos C, Soul JS, Gauvreau K, et al. Late gestation cerebellar growth is rapid and impeded by premature birth. *Pediatrics* 2005; **115**: 688-95.

45. Volpe JJ. Cerebellum of the premature infant: rapidly developing, vulnerable, clinically important. *J Child Neurol* 2009; **24**: 1085-104.

46. Limperopoulos C, Bassan H, Gauvreau K, et al. Does cerebellar injury in premature infants contribute to the high prevalence of long-term cognitive, learning, and behavioral disability in survivors? *Pediatrics* 2007; **120**: 584-93.

47. Limperopoulos C, Chilingaryan G, Sullivan N, Guizard N, Robertson RL, du Plessis AJ. Injury to the premature cerebellum: outcome is related to remote cortical development. *Cereb Cortex* 2014; **24**: 728-36.

48. Guo T, Duerden EG, Adams E, et al. Quantitative assessment of white matter injury in preterm neonates: Association with outcomes. *Neurology* 2017; **88**: 614-22.

49. Rand KM, Austin NC, Inder TE, Bora S, Woodward LJ. Neonatal Infection and Later Neurodevelopmental Risk in the Very Preterm Infant. *J Pediatr* 2016; **170**: 97-104.

50. Romeo DM, Ricci D, Brogna C, Mercuri E. Use of the Hammersmith Infant Neurological Examination in infants with cerebral palsy: a critical review of the literature. *Dev Med Child Neurol* 2016; **58**: 240-5.

51. Maitre NL, Chorna O, Romeo DM, Guzzetta A. Implementation of the Hammersmith Infant Neurological Examination in a High-Risk Infant Follow-Up Program. *Pediatr Neurol* 2016; **65**: 31-8.

52. Romeo DM, Cioni M, Palermo F, Cilauro S, Romeo MG. Neurological assessment in infants discharged from a neonatal intensive care unit. *Eur J Paediatr Neurol* 2013; **17**: 192-8.

53. Dyet LE, Kennea N, Counsell SJ, et al. Natural history of brain lesions in extremely preterm infants studied with serial magnetic resonance imaging from birth and neurodevelopmental assessment. *Pediatrics* 2006; **118**: 536-48.

54. Kapellou O, Counsell SJ, Kennea N, et al. Abnormal cortical development after premature birth shown by altered allometric scaling of brain growth. *PLoS Med* 2006; **3**: e265.

55. Rathbone R, Counsell SJ, Kapellou O, et al. Perinatal cortical growth and childhood neurocognitive abilities. *Neurology* 2011; **77**: 1510-7.

56. Kersbergen KJ, Benders MJ, Groenendaal F, et al. Different patterns of punctate white matter lesions in serially scanned preterm infants. *PLoS One* 2014; **9**: e108904.

57. Pavaine J, Young JM, Morgan BR, Shroff M, Raybaud C, Taylor MJ. Diffusion tensor imaging-based assessment of white matter tracts and visual-motor outcomes in very preterm neonates. *Neuroradiology* 2016; **58**: 301-10.

58. Cui J, Tymofiyeva O, Desikan R, et al. Microstructure of the Default Mode Network in Preterm Infants. *AJNR Am J Neuroradiol* 2017; **38**: 343-8.

59. Tin W, Fritz S, Wariyar U, Hey E. Outcome of very preterm birth: children reviewed with ease at 2 years differ from those followed up with difficulty. *Arch Dis Child Fetal Neonatal Ed* 1998; **79**: F83-7.

60. Roberts G, Howard K, Spittle AJ, Brown NC, Anderson PJ, Doyle LW. Rates of early intervention services in very preterm children with developmental disabilities at age 2 years. *J Paediatr Child Health* 2008; **44**: 276-80.

61. Janvier A, Barrington K. Trying to predict the future of ex-preterm infants: who benefits from a brain MRI at term? *Acta Paediatr* 2012; **101**: 1016-7.

62. Morgan C, Novak I, Dale RC, Guzzetta A, Badawi N. GAME (Goals - Activity - Motor Enrichment): protocol of a single blind randomised controlled trial of motor training, parent education and environmental enrichment for infants at high risk of cerebral palsy. *BMC Neurol* 2014; **14**: 203.

63. Guzzetta A, Boyd RN, Perez M, et al. UP-BEAT (Upper Limb Baby Early Action-observation Training): protocol of two parallel randomised controlled trials of action-observation training for typically developing infants and infants with asymmetric brain lesions. *BMJ Open* 2013; **3**.

64. Sgandurra G, Bartalena L, Cioni G, et al. Home-based, early intervention with mechatronic toys for preterm infants at risk of neurodevelopmental disorders (CARETOY): a RCT protocol. *BMC Pediatr* 2014; **14**: 268.

65. Spittle AJ, Barton S, Treyvaud K, Molloy CS, Doyle LW, Anderson PJ. School-Age Outcomes of Early Intervention for Preterm Infants and Their Parents: A Randomized Trial. *Pediatrics* 2016; **138**.

66. Spittle AJ, Anderson PJ, Lee KJ, et al. Preventive care at home for very preterm infants improves infant and caregiver outcomes at 2 years. *Pediatrics* 2010; **126**: e171-8.

67. Bosanquet M, Copeland L, Ware R, Boyd R. A systematic review of tests to predict cerebral palsy in young children. *Dev Med Child Neurol* 2013; **55**: 418-26.

68. Skiold B, Eriksson C, Eliasson AC, Aden U, Vollmer B. General movements and magnetic resonance imaging in the prediction of neuromotor outcome in children born extremely preterm. *Early Hum Dev* 2013; **89**: 467-72.

69. Romeo DM, Guzzetta A, Scoto M, et al. Early neurologic assessment in preterm-infants: integration of traditional neurologic examination and observation of general movements. *Eur J Paediatr Neurol* 2008; **12**: 183-9.

70. Setanen S, Lahti K, Lehtonen L, et al. Neurological examination combined with brain MRI or cranial US improves prediction of neurological outcome in preterm infants. *Early Hum Dev* 2014; **90**: 851-6.

71. Duerden EG, Guo T, Dodbiba L, et al. Midazolam dose correlates with abnormal hippocampal growth and neurodevelopmental outcome in preterm infants. *Ann Neurol* 2016; **79**: 548-59.

72. Young JM, Powell TL, Morgan BR, et al. Deep grey matter growth predicts neurodevelopmental outcomes in very preterm children. *Neuroimage* 2015; **111**: 360-8.

73. Kersbergen KJ, de Vries LS, Groenendaal F, et al. Corticospinal Tract Injury Precedes Thalamic Volume Reduction in Preterm Infants with Cystic Periventricular Leukomalacia. *J Pediatr* 2015; **167**: 260-8 e3.

74. Xu D, Bonifacio SL, Charlton NN, et al. MR spectroscopy of normative premature newborns. *J Magn Reson Imaging* 2011; **33**: 306-11.

75. Chawla NV, Bowyer KW, Hall LO, Kegelmeyer WP. SMOTE: synthetic minority over-sampling technique. *Journal of artificial intelligence research* 2002; **16**: 321-57.

76. Colby JB, Soderberg L, Lebel C, Dinov ID, Thompson PM, Sowell ER. Along-tract statistics allow for enhanced tractography analysis. *Neuroimage* 2012; **59**: 3227-42.

77. 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.

78. Raffelt DA, Tournier J-D, Smith RE, et al. Investigating white matter fibre density and morphology using fixel-based analysis. *Neuroimage* 2017; **144**: 58-73.

79. Tymofiyeva O, Hess C, Xu D, Barkovich A. Structural MRI connectome in development: challenges of the changing brain. *The British journal of radiology* 2014; **87**: 20140086.

80. Fornito A, Bullmore ET. Connectomics: a new paradigm for understanding brain disease. *Eur Neuropsychopharmacol* 2015; **25**: 733-48.

81. Tournier JD, Calamante F, Connelly A. Robust determination of the fibre orientation distribution in diffusion MRI: non-negativity constrained super-resolved spherical deconvolution. *Neuroimage* 2007; **35**: 1459-72.

82. Tuch DS. Q-ball imaging. *Magn Reson Med* 2004; **52**: 1358-72.

83. Zhang H, Schneider T, Wheeler-Kingshott CA, Alexander DC. NODDI: practical in vivo neurite orientation dispersion and density imaging of the human brain. *Neuroimage* 2012; **61**: 1000-16.

84. Fieremans E, Jensen JH, Helpern JA. White matter characterization with diffusional kurtosis imaging. *Neuroimage* 2011; **58**: 177-88.

85. Jeurissen B, Tournier JD, Dhollander T, Connelly A, Sijbers J. Multi-tissue constrained spherical deconvolution for improved analysis of multi-shell diffusion MRI data. *Neuroimage* 2014; **103**: 411-26.

86. Jelescu IO, Veraart J, Adisetiyo V, Milla SS, Novikov DS, Fieremans E. One diffusion acquisition and different white matter models: how does microstructure change in human early development based on WMTI and NODDI? *Neuroimage* 2015; **107**: 242-56.

87. Kunz N, Zhang H, Vasung L, et al. Assessing white matter microstructure of the newborn with multi-shell diffusion MRI and biophysical compartment models. *Neuroimage* 2014; **96**: 288-99.

88. Shi J, Chang L, Wang J, et al. Initial Application of Diffusional Kurtosis Imaging in Evaluating Brain Development of Healthy Preterm Infants. *PLoS One* 2016; **11**: e0154146.

Appendices

Appendix 1-2: Ethics Approvals for the PPREMO study

Appendix 1: Royal Brisbane and Women's Hospital Human Research Ethics Committee Approval: HREC/12/QRBW/245 (10/09/12)

Appendix 2: The University of Queensland Human Research Ethics Committee Approval: 2012001060 (20/09/12)

Appendix 3-5: Parent/guardian information statements and consent forms

Appendix 3: Parent Information and Consent Form (PICF) – Preterm group

Appendix 4: Parent Information and Consent Form (PICF) – Term group

Appendix 5: Audio-visual Consent Form

Appendix 6-7: Recruitment Flyers

Appendix 6: Study Flyer – Preterm group

Appendix 7: Study Flyer – Term group

Appendix 8: Protocol paper Additional File 1: PPREMO questionnaire

Appendix 9: Protocol paper Additional File 2: MR Protocol

Appendix 10: Structured Neurological Assessment for Paediatrician review at 12 months corrected age

Royal Brisbane and Women's Hospital
Metro North Hospital and Health Service

Queensland Government

Office of the Human Research Ethics Committees

Queensland **Health**

Enquiries to: Odette Petersen Coordinator
Phone: 07 3646 5490
Fax: 07 3646 5849
Our Ref: HREC/12/QRBW/245
E-mail RBWH-Ethics@health.qld.gov.au

Professor Paul Colditz
Professor of Perinatal Research Centre
Level 6
Ned Hanlon Building

Dear Professor Colditz

Re: Ref Nº: HREC/12/QRBW/245 Relationship between brain structure and function of very premature infants to predict neurodevelopmental outcome

Thank you for submitting the above research project for single ethical review. This project was considered by the Royal Brisbane & Women's Hospital Human Research Ethics Committee (RBWH HREC) **(EC00172)** meeting held on 20.08.12.

I am pleased to advise that the RBWH Human Research Ethics Committee has granted ethical approval of this research project.

The nominated participating sites in this project are:

- Royal Brisbane & Women's Hospital, QLD

Note: If additional sites are engaged prior to the commencement of, or during the research project, the Coordinating Principal Investigator is required to notify the RBWH HREC. Notification of withdrawn sites should also be provided to the RBWH HREC in a timely fashion.

This letter constitutes ethical approval only. This project cannot proceed at any site until separate research governance authorisation has been obtained from the CEO or Delegate of the institution under whose auspices the research will be conducted at that site.

This HREC is constituted and operates in accordance with the National Health and Medical Research Council's (NHMRC) National Statement on Ethical Conduct in Human Research (2007). The processes used by this HREC to review multi-centre research proposals have been certified by the National Health and Medical Research Council.

- In accordance with Section 3.3.22 (b) of the National Statement the Coordinating Principal Investigator will report to the RBWH HREC annually in the specified format and notify the HREC when the project is completed at all sites, the first report being due on **10.09.13** and a final report is to be submitted on completion of the study. These instructions can be found at http://www.health.qld.gov.au/ohmr/html/regu/reporting_templates.asp.

- The Coordinating Principal Investigator will notify the RBWH HREC if the project is discontinued at a participating site before the expected completion date, with reasons provided.

- The Coordinating Principal Investigator will notify the RBWH HREC of any plan to extend the duration of the project past the approval period listed above and will submit any associated required documentation. Instructions for obtaining an extension of approval can be found at http://www.health.qld.gov.au/rbwh/research/hrec.asp.

- The Coordinating Principal Investigator will notify the RBWH HREC of his or her inability to continue as Coordinating Principal Investigator including the name of and contact information for a replacement.

- A copy of this ethical approval letter together with completed Site Specific Assessment (SSA) and any other requirements must be submitted by all site Principal Investigators to the Research Governance Office at each participating institution in a timely manner to enable the institution to authorise the commencement of the project at its site/s.

- Should you have any queries about the RBWH HREC's consideration of your project please contact the HREC Coordinator on 07 3646 5490. The RBWH HREC's Terms of Reference, Standard Operating Procedures, membership and standard forms are available from http://www.health.qld.gov.au/rbwh/research/hrec.asp.

The RBWH HREC wishes you every success in your research.

Yours sincerely

Dr Conor Brophy
Chairperson RBWH Human Research Ethics Committee
Metro North Hospital and Health Service
10.09.2012

This HREC is constituted and operates in accordance with the National Health and Medical Research Council's (NHMRC) *National Statement on Ethical Conduct in Human Research (2007)*. The processes used by this HREC to review multi-centre research proposals have been certified by the National Health and Medical Research Council.

THE UNIVERSITY OF QUEENSLAND

Institutional Approval Form For Experiments On Humans Including Behavioural Research

Chief Investigator:	Prof Paul Colditz, Prof Roslyn Boyd, Mrs Joanne George, Dr Raymond Chuk, A/Prof Stephen Rose, Mrs Sonia Sam, Ms Kerstin Pannek, Mrs Rebecca Caesar, Dr Robert Ware
Project Title:	Relationship Between Brain Structure And Function Of Very Premature Infants To Predict Neurodevelopmental Outcome PPREMO (Prediction Of Preterm Motor Outcomes)
Supervisor:	Prof Roslyn Boyd, Prof Paul Colditz, A/Prof Stephen Rose, Dr Barbara Lingwood
Co-Investigator(s):	Ms Karen Taylor, Dr Barbara Lingwood
Department(s):	UQ School of Medicine, Perinatal Research Centre RBWH
Project Number:	2012001060
Granting Agency/Degree:	CP Alliance
Duration:	1st August 2016

Comments:

Expedited review on the basis of approval from the Royal Brisbane and Women's Hospital HREC, dated 10/09/2012.

Name of responsible Committee:-
Medical Research Ethics Committee
This project complies with the provisions contained in the *National Statement on Ethical Conduct in Human Research* and complies with the regulations governing experimentation on humans.

Name of Ethics Committee representative:-
Professor Bill Vicenzino
Chairperson
Medical Research Ethics Committee

Date: 20/9/12 Signature: _____

THE UNIVERSITY OF QUEENSLAND AUSTRALIA

Queensland Government

Parent Information and Consent Form (PICF) **(Version 5; 14/05/14)**

Title of the Research study: Prediction of PREterm Motor Outcomes (PPREMO)

Thank you for taking the time to read the information in this form. These pages contain information about a research project we are inviting you and your baby to take part in. The purpose of this information is to explain to you clearly and openly all the steps and procedures of this project. The information is to help you to decide whether or not you would like to take part in the study. Please read this information carefully. You can ask us questions if you wish. You may also wish to talk about the project with others e.g. friends or a health care worker. When you understand what the project is about, you can sign the consent form attached if you agree to take part. You will be given a copy of this PICF to keep.

What is the Research Project about?

This project is for babies born before 31 weeks gestation (preterm). Some babies who are born preterm can have problems later in life (for example with learning, movement or behaviour). It is difficult to know which babies will have problems and which babies won't. This makes it difficult for doctors to know which babies will need extra help with their development of learning and movement skills.

Magnetic Resonance Imaging (MRI) scans of your baby's brain can be performed safely at an earlier stage (30 weeks) than has been possible in the past (at term). MRI's are safe and provide information about your baby's brain and how it compares to babies born at term. Early brain scans are one of the tests we will be investigating in this study.

The purpose of this research project is to learn which tests (clinical and MRI) can be used at 30 weeks and 40 weeks, to accurately identify which babies may have problems later in life, so that those babies and their families can be provided with the help they need as early as possible.

Why are you being invited to be in the study?

We are inviting all parents/guardians of babies born at less than 31 weeks in this hospital to participate in the study. We will also invite some babies born at term to serve as a comparison.

What are your alternatives to participating in this project?

There is no obligation to participate in this study. Should you choose not to participate in this project you and your baby will have all the usual access to treatment.

What does this study involve?

There are 4 stages of this study:

At 30-36 weeks of age, while your baby is still in the nursery we will carry out the following:

- clinical and medical information will be collected from the baby's chart
- Video of their movements in their incubator or cot (up to 1 hour of video; no handling of the baby)
- A neurological/neurobehavioural assessment (15-20 minutes; involves a small amount of handling).This assessment is videoed for scoring purposes.
- A brain scan (MRI) which takes about an hour (20 minutes preparation and 40 minutes in the scanner)

At 40 weeks which is term equivalent age; if you have returned home we will ask you to visit the hospital. We will complete:

- A video of their movements for a short period (up to 15 minutes)
- Movement assessments and a neurological assessment (30-40 minutes).This assessment is videoed for scoring purposes.
- Assessment of their visual functions (5 minutes)
- A brain scan (MRI) which takes up to one hour (20 minutes preparation and 40 minutes in the scanner)
- A recording of their brain's electrical activity (EEG; 30 minutes preparation and 30 minutes recording).
- Ask you to complete a brief questionnaire of social and environmental factors that relate to your family

At 12 weeks corrected age (3 months after term); we will visit you at home and:

- Video your baby's movements for a short period (5-15 minutes)
- Perform a movement assessment (40 minutes). This assessment is videoed for scoring purposes.
- Assess their visual function (5 minutes)

At 12 months corrected age; we will ask you to visit the hospital:

- A paediatrician will assess your baby's general development (30 minutes)
- We will perform movement assessments (11/2 hours). These assessments are videoed for scoring purposes.

What is an MRI and what does it involve?

A magnetic resonance scan (MRI) involves your baby being transported by a doctor and a nurse from the nursery to the MRI facility on the 3rd floor of the Royal Brisbane and Women's hospital in a special incubator that allows similar monitoring to that in the nursery. MRI is safe, there is no radiation, it has no known dangerous or harmful effects, causes no pain, and no sedation or any drugs are given to your baby.

The MRI will be performed in the same way as for all babies who require MRI in the hospital. You will be provided with an MRI Fact Sheet that is made available to parents of all babies having an MRI in the hospital. The scanner will take pictures of your infant's brain using magnetic and radio waves. No medications or X-rays are used, there is no radiation involved and there is no potential for harm. Before the scan your baby will be fed in the usual way to encourage him/her to sleep during the scan. He/she will then be positioned in a comfortable pillow in the scanner and monitored over the scan time (approximately 40 minutes). During the scan most infants sleep as it is after a feed.

Ear muffs will be placed over their ears as part of the MRI scan is noisy. A sensor will be placed on the hand or foot to monitor heart rate and oxygen levels as a safety measure, because during the MRI the baby is not clearly visible. A doctor and a nurse from the Neonatal Nursery will be with the baby at all times to monitor the baby and the Hospital has an established emergency protocol to follow in the unlikely event that vomiting or apnoea (stopping breathing) occurs. MRI does not increase the risk of these events which can happen to any baby at any time.

What is an EEG and what does it involve?

EEG is a standard method to measure brain waves in babies. It involves placing a cap on your baby's head that contains a number of small sponges. This does not hurt your baby and there is no potential for harm. Brain waves are recorded to a computer. The recording, which lasts for up to about 30 minutes can be made regardless of whether your baby is asleep or awake.

What is the Neonatal assessment of visual functions?

When your baby is alert, we will test how your baby looks at (fixes and follows) a series of cards designed to test their vision.

What are neurological and neurobehavioural assessments?

These assessments look at how your baby is developing their skills of movement and posture, and how they are interacting with and responding to their environment. The researcher will move your baby's arms and legs, test their reflexes, place them in different positions such as on their tummy and back and observe their movements, and provide some stimulation such as shaking a rattle or bell and observing how they respond. These assessments are video recorded for scoring purposes.

What is the Questionnaire I will be asked to complete?

Any child's development is influenced by both medical or biological factors (such as prematurity, illness etc.) and social or environmental factors (such as the home environment). The information you provide in the questionnaire is totally confidential, and will allow us to understand which information from our assessments is as a result of their prematurity. We are only investigating the biological or medical factors relating to prematurity.

Who are the Researchers?

Joanne George, a physiotherapist, leads the project and will perform all assessments on your baby. Other researchers involved in this project include: Professor Paul Colditz (a neonatologist and Professor of Perinatal medicine), Professor Roslyn Boyd (a physiotherapist), Associate Professor Stephen Rose and Kerstin Pannek (physicists' who will analyse your baby's MRI scan), Professor Alan Coulthard (a radiologist), Sonia Sam and Rebecca Caesar (physiotherapists who will also perform some motor assessments), Dr Barbara Lingwood (a scientist who may analyse some of the data), Karen Taylor (a research nurse), and Dr Robert Ware (a biostatistician who will analyse some of the data).

What are the benefits of participating in this study?

Additional assessments will be performed, compared to babies not in the study. The information from these assessments will be provided to your child's doctor who will pass the information on to you in your regular appointments. You will have the opportunity to gain a set of MRI scan films of your infant's brain for the future record of your child. If any neurodevelopmental issues arise when your child is older, the MRI scans may be helpful. You will have the opportunity to discuss your child's progress in depth and discuss any concerns with experienced staff. You will have an

opportunity for in depth neurodevelopmental assessments at 40 weeks (term), as well as at 12 weeks post term and 12 months of age.

Is there likely to be a benefit to other babies in the future?

If MRI and/or movement assessments performed at 30 weeks and 40 weeks are shown to be accurate in terms of predicting movement development at 1 year of age, then this finding will benefit many babies in the future. If future practice is made better, this may benefit other premature babies in the future.

What are the possible risks and/or side effects for my baby?

There are no anticipated risks to your baby as a result of being part of this research project. However if any risks become evident at any time, we will let you know immediately.

There are no known risks of Magnetic Resonance Imaging. MRI is commonly done for research purposes for infants born preterm. Most infants will sleep or rest during the scan. If your baby becomes distressed for any reason the study will be stopped. Your baby will be monitored carefully throughout the scan by trained medical and/or nursing staff.

There is the possibility that the MRI scan will show up something in your infant's brain that we had not expected. If this happens, we will arrange for you to meet with a medical professional who can explain the findings to you. If any of the results of the MRI, or neurodevelopmental assessments, are distressing for you we will arrange specific counseling to discuss the findings with specially trained staff. Although detecting a significant brain abnormality is extremely unlikely, you should be aware that if an abnormality is detected in your child and you are told about it, then this knowledge may have consequences for your child. Knowing about an abnormality may affect their ability to work in certain professions, obtain life or health insurance and other facets of daily living; however you should be aware that this is unlikely. Please take the time to consider carefully what it would mean if we told you your child had an abnormality in their brain that might, or might not, affect your child in later life. If you do not wish to know this, then you may wish to discuss this further before agreeing to participate. You can choose to participate in the study but not receive information from the scans and movement assessments.

What are the possible discomforts and/or inconveniences for my baby or me?

The inconvenience to you and your baby is the time that the assessments will take, and the trips you will need to make to the hospital. Families will have to make between 1 and 2 trips to the hospital

for the assessments. We will make the appointments at a time that suits you and provide some compensation for travel costs and parking.

The MRI scanner is noisy, so protective earmuffs will be positioned over your infant's ears during the scan.

What will be done to make sure the information is confidential?

All results of all assessments will be stored without your child's name on it. All hard copy data will be stored in a secure filing cabinet and only the researchers will have access to these. Video will only be viewed by study personnel for the purposes of data collection and assessment scoring. If we talk or write about the results of this research, we will not use any names. All data is only accessible to the study personnel.

Queensland Health guidelines require the storage of research data involving minors to be kept for 15 years after the child has turned 18 years of age.

As is regular procedure in infant studies, the name of the family GP will be collected in order to allow direct sharing of information and concerns regarding potential risks for the child if necessary.

Will I be informed of the results when the research project is finished?

A regular 6 monthly newsletter will also be sent to you to keep you updated on study recruitment and progress. At the conclusion of the study all families will be sent a meaningful summary of the overall study results, and copies of publications if requested.

Participation in future research

In the consent form we will ask you if you agree to be contacted in the future if further follow up studies are developed. Your consent to be contacted would only apply to extended research which relates to the current research project. Full ethical approval would be sought by the research team and a new consent process undertaken. You can choose to participate in this study but decline to be contacted for future research.

Participation in this study is voluntary

You can decide whether or not you wish to take part in this research project. You can decide to withdraw from this research project at any time. No explanation is needed. You may like to discuss your participation in this research project with your family and/or with your doctor. You can ask for further information before deciding if your child will take part.

If you would like more information about the study or if you need to contact a study representative in an emergency, the person to contact is:

Professor Paul Colditz,

Royal Brisbane and Women's Hospital

Contact telephone: (07) 3346 6014

This study has been reviewed and approved by the Royal Brisbane & Women's Hospital, Human Research Ethics Committee (HREC). Should you wish to discuss the study in relation to your rights as a participant or should you wish to make an independent complaint, you may contact the Coordinator or Chairperson, Human Research Ethics Committee, Royal Brisbane & Women's Hospital, Herston, Qld 4029 or telephone (07) 3646 5490, email Ethics@health.qld.gov.au

STANDARD INFORMED CONSENT
FOR PARENTS / GUARDIANS TO GIVE CONSENT
FOR THEIR BABY TO PARTICIPATE IN A RESEARCH PROJECT

Title of Project: Prediction of Preterm Early Motor Outcomes (PPREMO)

Principal and associate Investigators	Professor Paul Colditz, Professor Roslyn Boyd, Joanne George, , Sonia Sam, A/Prof Stephen Rose, Kerstin Pannek, Professor Alan Coulthard, Rebecca Caesar, Karen Taylor, Dr Barbara Lingwood, Dr Robert Ware

I/We (Parents/Guardians name) _____

Parents/Guardians of (baby's name) _____

voluntarily consent to him / her taking part in the above titled Research Project, explained to me by

Mr / Ms / Dr / Professor

1. I/We have received a Participant Information and Consent Form (PICF) to keep and I/we understand the purpose, extent and possible effects of my baby's involvement
1. I/We have been asked if I/we would like to have a family member or friend with me/us while the project is explained
2. I/We have had the opportunity to ask questions and I/we am/are satisfied with the answers I/we have received/We understand that the researcher has agreed not to reveal results of any information involving my/our baby, subject to legal requirements
3. I/We understand that the name of our family GP will be collected in order to allow direct sharing of information and concerns regarding potential risks for the child if necessary.
4. I/We agree to video recording of assessments for data collection and scoring purposes.
5. If information about this project is published or presented in any public form, I/we understand that the researcher will not reveal my/our baby's identity.
6. I/We understand that if I/we refuse to consent, or if I/we withdraw my/our baby from the study at any time with or without explanation, this will not affect my/our baby's access to the standard treatment that all babies receive.
7. I/We agree to be contacted in future if a further research study is planned. Yes ☐ No ☐
    - I/We understand I/we will receive a copy of this consent form.

	Printed Name	Signature	Date
PARENT/GUARDIAN 1			
PARENT/GUARDIAN 2			

I have explained the study to the parents/guardians who has signed above, and believe that they understand the purpose, extent and possible effects of their involvement in this study.

Printed Name _____ Signature _____ Date _____

RESEARCHER _____ _____

Note: All parties signing the Consent Form must date their own signature.

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

Queensland Government

Parent Information and Consent Form (PICF) (Version 5; 14/05/14)

Title of the Research study: Prediction of PREterm Motor Outcomes (PPREMO)

Thank you for taking the time to read the information in this form. These pages contain information about a research project we are inviting you and your baby to take part in. The purpose of this information is to explain to you clearly and openly all the steps and procedures of this project. The information is to help you to decide whether or not you would like to take part in the study. Please read this information carefully. You can ask us questions if you wish. You may also wish to talk about the project with others e.g. friends or a health care worker. When you understand what the project is about, you can sign the consent form attached if you agree to take part. You will be given a copy of this PICF to keep.

What is the Research Project about?

Some babies who are born prematurely can have problems later in life (for example with learning, movement or behaviour). It is difficult to know which babies will have problems and which babies won't. This makes it difficult for doctors to know which babies will need extra help with their development of learning and movement skills.

We are investigating whether early brain scans and other assessments can help us accurately identify which babies may have problems later in life, so that those babies and their families can be provided with the help they need as early as possible. To do this we also need brain scans of healthy infants born at full term as a comparison.

Why are you being invited to be in the study?

We are looking for healthy babies born at full term to act as a reference group for our study. By having information and brain scans of healthy babies, it will help us to understand the brain scans of the premature infants.

Who is eligible for this study?

1. Healthy infants born between 38 and 41 weeks gestation.
2. Infants may not be growth restricted i.e. must be above the 10th percentile on the growth chart at birth.
3. Infants may not have been admitted to the nursery after birth.
4. English speaking families as we don't have access to interpreters.
5. Families need to be available to attend the hospital for a visit when their baby is 1 week old.

What are your alternatives to participating in this project?

There is no obligation to participate in this study. Should you choose not to participate in this project you and your baby will have all the usual medical care.

What does this study involve?

When your baby is 1 week old we will ask you to visit the hospital where we will perform:

- A brain scan (MRI) which takes up to one hour (20 minutes preparation and 40 minutes in the scanner)
- A recording of their brain's electrical activity (EEG; 30 minutes preparation and 30 minutes recording).
- Assessment of their visual functions (5 minutes)
- A neurological/neurobehavioural assessment (20minutes). This assessment is videoed for data collection and scoring purposes.
- Ask you to complete a confidential questionnaire

What is an MRI and what does it involve?

A magnetic resonance scan is a brain scan that takes pictures of your baby's brain. It is safe, there is no radiation, it has no known dangerous or harmful effects, causes no pain, and no sedation or any drugs are given to your baby.

The MRI will be performed in the same way as for all babies who require MRI in the hospital. You will be provided with an MRI Fact Sheet that is made available to parents of all babies having an MRI in the hospital. The scanner will take pictures of your infant's brain using magnetic and radio waves. No medications or X-rays are used, there is no radiation involved and there is no potential for harm. Before the scan your baby will be fed in the usual way to encourage him/her to sleep during the scan. He/she will then be positioned in a comfortable pillow in the scanner and

monitored over the scan time (approximately 40 minutes). During the scan most infants sleep as it is after a feed.

Ear muffs will be placed over their ears as part of the MRI scan is noisy. A sensor will be placed on the hand or foot to monitor heart rate and oxygen levels as a safety measure, because during the MRI the baby is not clearly visible. A doctor from the Neonatal Nursery will be with the baby at all times to monitor the baby.

What is an EEG and what does it involve?

EEG is a standard method to measure brain waves in babies. It involves placing a cap on your baby's head that contains electrodes in the form of small sponges. This does not hurt your baby and there is no potential for harm. Brain waves are recorded to a computer. The recording, which lasts for up to about 30 minutes can be made regardless of whether your baby is asleep or awake.

What is the Neonatal assessment of visual functions?

When your baby is alert, we will test how your baby looks at (fixes and follows) a series of cards designed to test their vision.

What are neurological and neurobehavioural assessments?

These assessments look at how your baby is developing their skills of movement and posture, and how they are interacting with and responding to their environment. The researcher will move your baby's arms and legs, test their reflexes, place them in different positions such as on their tummy and back and observe their movements, and provide some stimulation such as shaking a rattle or bell and observing how they respond. These assessments are video recorded for scoring purposes.

What is the Questionnaire I will be asked to complete?

Any child's development is influenced by both medical or biological factors (such as prematurity, illness etc.) and social or environmental factors (such as the home environment). The information you provide in the questionnaire is totally confidential, and will allow us to understand which information from our assessments is as a result of biological factors or environmental factors. We are only investigating the biological or medical factors in this study. You can choose not to answer questions in the questionnaire.

Who are the Researchers?

Joanne George, a physiotherapist, leads the project and will perform all assessments on your baby. Other researchers involved in this project include: Professor Paul Colditz (a neonatologist and Professor of Perinatal medicine), Professor Roslyn Boyd (a physiotherapist), Associate Professor Stephen Rose and Kerstin Pannek (physicists' who will assess your baby's MRI scan), Professor Alan Coulthard (a radiologist), Sonia Sam and Rebecca Caesar (physiotherapists who will also perform some motor assessments), Dr Barbara Lingwood (a scientist who may analyse some of the data), Karen Taylor (a research nurse), and Dr Robert Ware (a biostatistician who will analyse some of the data).

What are the benefits of participating in this study?

You may request a report of the MRI which will be provided to your child's Paediatrician or GP.

Is there likely to be a benefit to other babies in the future?

If MRI and/or movement or neurological assessments performed at 30 weeks and 40 weeks are shown to be accurate in terms of predicting movement development at 1 year of age, then this finding will benefit many babies in the future. If future practice is made better, this may benefit other premature babies in the future.

What are the possible risks and/or side effects for my baby?

There are no anticipated risks to your baby as a result of being part of this research project. However if any risks become evident at any time, we will let you know immediately.

There are no known risks of Magnetic Resonance Imaging. Most infants will sleep or rest during the scan. If your baby becomes distressed for any reason the study will be stopped. Your baby will be monitored carefully throughout the scan by trained medical and/or nursing staff.

There is the possibility that the MRI scan will show up something in your infant's brain that we had not expected. If this happens, we will arrange for you to meet with a medical professional who can explain the findings to you. If any of the results of the MRI, or neurodevelopmental assessments, are distressing for you we will arrange specific counseling to discuss the findings with specially trained staff. Although detecting a significant brain abnormality is extremely unlikely, you should be aware that if an abnormality is detected in your child and you are told about it, then this knowledge may have consequences for your child. Knowing about an abnormality may affect their ability to work in certain professions, obtain life or health insurance and other facets of daily living,

however you should be aware that this is unlikely. Please take the time to consider carefully what it would mean if we told you your child had an abnormality in their brain that might, or might not, affect your child in later life. If you do not wish to know this, then you may wish to discuss this further before agreeing to participate. You can choose to participate in the study but not receive information from the scans and other assessments.

What are the possible discomforts and/or inconveniences for my baby or me?

The inconvenience to you and your baby is the time that the assessments will take, and the trip you will need to make to the hospital. We will make the appointment at a time that suits you and provide some compensation for travel costs and parking. The MRI scanner is noisy, so protective earmuffs will be positioned over your infant's ears during the scan.

What will be done to make sure the information is confidential?

All results of all assessments will be stored without your child's name on it. All hard copy data will be stored in a secure filing cabinet and only the researchers will have access to these. Video will only be viewed by study personnel for the purposes of data collection and assessment scoring. If we talk or write about the results of this research, we will not use any names. All data is only accessible to the study personnel.

Queensland Health guidelines require the storage of research data involving minors to be kept for 15 years after the child has turned 18 years of age. As is regular procedure in infant studies, the name of the family GP will be collected in order to allow direct sharing of information or concerns regarding potential risks for the child if necessary.

Participation in future research

In the consent form we will ask you if you agree to be contacted in the future if further follow up studies are developed. Your consent to be contacted would only apply to extended research which relates to the current research project. Full ethical approval would be sought by the research team and a new consent process undertaken. You can choose to participate in this study but decline to be contacted for future research.

Will I be informed of the results when the research project is finished?

If you would like to be informed of the study's progress a regular 6 monthly newsletter will be sent to you to keep you updated on study recruitment and progress. At the conclusion of the study all

families will be sent a meaningful summary of the overall study results, and copies of publications if requested.

Participation in this study is voluntary

You can decide whether or not you wish to take part in this research project. You can decide to withdraw from this research project at any time. No explanation is needed. You may like to discuss your participation in this research project with your family and/or with your doctor. You can ask for further information before deciding if your child will take part.

If you would like more information about the study or if you need to contact a study representative in an emergency, the person to contact is:

Professor Paul Colditz,

Royal Brisbane and Women's Hospital

Contact telephone: (07) 3346 6014

This study has been reviewed and approved by the Royal Brisbane & Women's Hospital, Human Research Ethics Committee (HREC). Should you wish to discuss the study in relation to your rights as a participant or should you wish to make an independent complaint, you may contact the Coordinator or Chairperson, Human Research Ethics Committee, Royal Brisbane & Women's Hospital, Herston, Qld 4029 or telephone (07) 3636 5490, email Ethics@health.qld.gov.au

STANDARD INFORMED CONSENT
FOR PARENTS / GUARDIANS TO GIVE CONSENT
FOR THEIR BABY TO PARTICIPATE IN A RESEARCH PROJECT

Title of Project - Prediction of Preterm Early Motor Outcomes (PPREMO)

Principal and associate Investigator(s)	Professor Paul Colditz, Professor Roslyn Boyd, Joanne George, Sonia Sam, A/Prof Stephen Rose, Kerstin Pannek, Rebecca Caesar, Karen Taylor, Dr Barbara Lingwood, Dr Robert Ware

I/We (Parents/Guardians name) _____

Parents/Guardians of (baby's name) _____

voluntarily consent to him / her taking part in the above titled Research Project, explained to me by

Mr / Ms / Dr / Professor

8. I/We have received a Participant Information and Consent Form (PICF) to keep and I/we understand the purpose, extent and possible effects of my baby's involvement
2. I/We have been asked if I/we would like to have a family member or friend with me/us while the project is explained
9. I/We have had the opportunity to ask questions and I/we am/are satisfied with the answers I/we have received/We understand that the researcher has agreed not to reveal results of any information involving my/our baby, subject to legal requirements
10. I/We understand that the name of our family GP will be collected in order to allow direct sharing of concerns regarding potential risks for the child if necessary.
11. I/We agree to video recording of assessments for data collection and scoring purposes.
12. If information about this project is published or presented in any public form, I/we understand that the researcher will not reveal my/our baby's identity.
13. I/We understand that if I/we refuse to consent, or if I/we withdraw my/our baby from the study at any time with or without explanation, this will not affect my/our baby's access to the standard treatment that all babies receive.
14. I/We agree to be contacted in future if a further research study is planned. Yes ☐ No ☐
15. I/We understand I/we will receive a copy of this consent form.
16. I/We have been asked if we wish to have a report of the MRI sent to our baby's doctor.
    Yes, I would like a report ☐ No thanks ☐ Doctor's name:

	Printed Name	Signature	Date
PARENT/GUARDIAN 1			
PARENT/GUARDIAN 2			

I have explained the study to the parents/guardians who has signed above, and believe that they understand the purpose, extent and possible effects of their involvement in this study.

Printed Name _____ Signature _____ Date _____

RESEARCHER

_____ _____

Note: All parties signing the Consent Form must date their own signature.

PPREMO

(Prediction of Preterm Motor Outcomes)

Audio-visual/Photographic/Media Consent Form (Version 2; 20/9/13)

The PPREMO research team would like your permission to take images or make audio-visual recordings of your child for the PPREMO research study assessments.

These images or recordings will be stored carefully and sensitively. They may be reused for the purposes of:

1. Research records – they will be used by the researchers to make assessments.

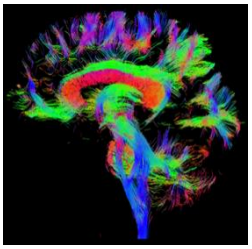
2. Education and training – they may be used in presentations at medical conferences, seminars or lectures where information is shared amongst health professionals for teaching purposes. The identity of your child will be protected at all times.

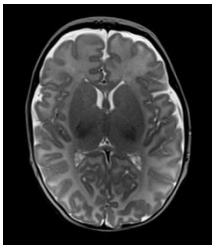
3. Publications – they may be used in medical journals, on medical conference posters, in health professional newsletters, reports or brochures, and on restricted access internet pages for health professionals. The identity of your child will be protected at all times.

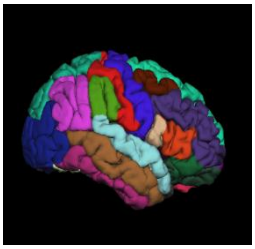
If you change your mind at any time, you are welcome to contact a research team member to withdraw your consent.

If you would like more information about the use of imaging/videos in the study or need to contact a study representative, please contact:

Ms Joanne George
Royal Brisbane & Women's Hospital
Ph.: (07) 3646 9609







STANDARD INFORMED CONSENT
FOR PARENTS / GUARDIANS TO GIVE CONSENT
FOR IMAGES/VIDEO TO BE RECORDED OF THEIR CHILD AS PART OF THE PPREMO STUDY

Title of Project: Prediction of Preterm Early Motor Outcomes (PPREMO)

I/We (Parent's/Guardian's name) _____

Parent's/Guardians of (babies' name/s) _____

consent to him / her taking part in the above, explained to me by

Mr / Ms / Dr / Professor

(please initial in the box to consent)

17. I/We have received an Audio-visual/Photographic/Media Consent Form to keep and I/we give consent for images/video to be taken of our child for the PPREMO study. ☐

18. I/We consent to the video recordings being used for assessments. ☐

19. I/We consent to the images and video recordings to be used for education and training purposes in medical conferences, seminars, lectures for teaching health professionals. I understand that the identity of my/our child will be protected at all times. ☐

20. I/We consent to the images and video recordings to be used in publications, such as medical journals, on medical conference posters, in health professional newsletters, reports and brochures, and on restricted access internet pages for health professionals. I understand that the identity of my/our child will be protected at all times. ☐

21. I/We understand that we can withdraw consent at any time without affecting our standard of care ☐

	Printed Name	Signature	Date
PARENT/GUARDIAN 1			
PARENT/GUARDIAN 2			

I have explained the reason for this consent to the parents/guardians who has signed above, and believe that they understand the purpose, extent and possible effects of this consent.

Printed Name _____ Signature _____ Date _____

RESEARCHER _____ _____

Note: All parties signing the Consent Form must date their own signature.

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

Queensland Government

PPREMO (Prediction of PREterm Motor Outcomes)

What is this study about? Learning new ways to identify which premature babies need extra help with their development. New, safe, brain scan technology is available and we aim to learn how these brain scans can help identify babies who may need help, at an earlier stage.

How can you help? If your baby was born before 31 weeks gestation we would like to discuss with you whether you would like to participate in the study.

What do you need to do? Ask your doctor about the study and whether we can come and talk to you about it.

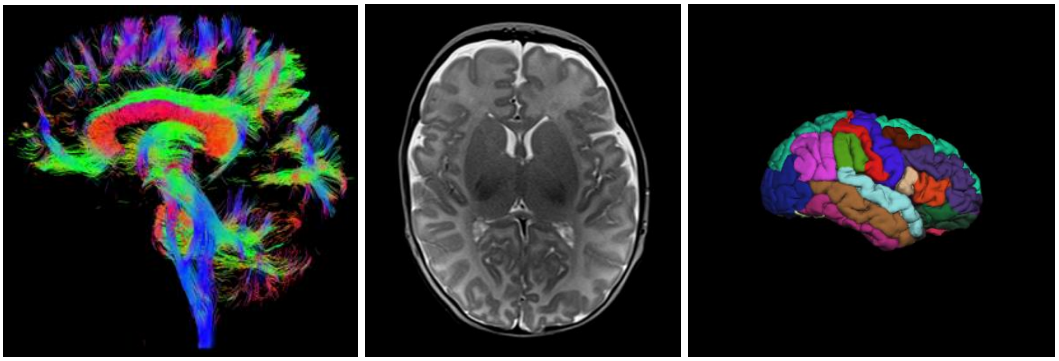
Benefits:

- your baby will have additional assessments to check their development which would not be available to babies not in the study. This information will be given to your doctor to feed back to you in your regular appointments.
- you will be assisting us to gather information that may improve the care of premature babies and provide better outcomes for their future.

If you would like to find out more or know someone who might be interested, please contact:

Professor Paul Colditz **(Principal Investigator)**
ph.: **3346 6014** mob: **0416290018** email: **p.colditz@uq.edu.au**

Joanne George **(Principal Investigator)**
ph.: **3646 9609** mob: **0423968680** email: **j.george2@uq.edu.au**



THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

Queensland Government

Healthy babies to be a reference group
For our PPREMO study:
Prediction of PREterm Motor Outcomes

What is this study about?

Learning new ways to identify which premature babies need extra help with their development. New, safe, brain scan technology is available and we aim to learn how these brain scans can help identify babies who may need help, at an earlier stage.

How can you help?

We need healthy babies born at term to act as a reference group to help us understand our research findings. It involves one visit to the hospital when your baby is 1 week old.

What do you need to do?

Contact us using the details below if your baby meets the following criteria:

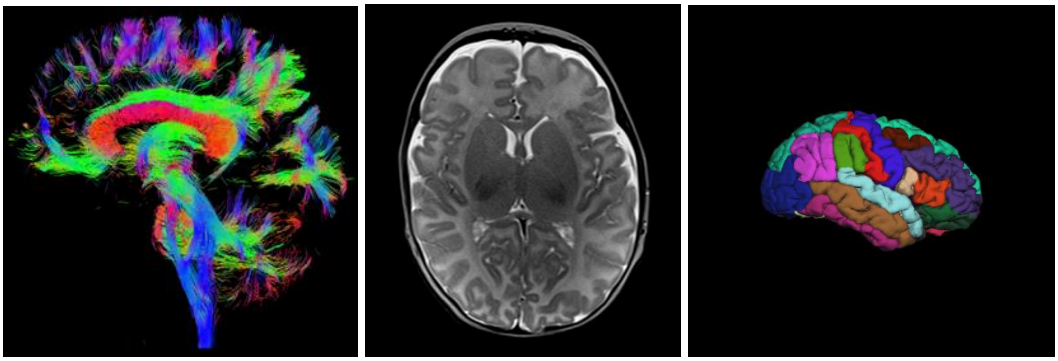
1. Healthy baby born between 38 and 41 weeks gestation
2. English speaking family as we have no interpreters
3. Able to visit the hospital for a few hours when your baby is 1 week old

Benefits:

You will be assisting us to gather information that may improve the care of premature babies and provide better outcomes for their futures.

If you would like to find out more or know someone who might be interested, please contact:

Professor Paul Colditz **(Principal Investigator)**
ph.: **3346 6014** mob: **0416290018** email: **p.colditz@uq.edu.au**
Joanne George **(Principal Investigator)**
ph.: **3646 9609** mob: **0423968680** email: **j.george2@uq.edu.au**



(Version 2; 25/7/13)

PPREMO

Prediction of PREterm Motor Outcomes

Questionnaire for the

Mother or Primary caregiver

STUDY NUMBER: | | | |

TODAY'S DATE: | | | | | | |
D D M M Y Y

INSTRUCTIONS:

This questionnaire is designed for the mother, or primary caregiver, of the preterm child in our study. It contains a number of questions about you and your family. All your responses are totally confidential and only members of the research team will have access to this information.

Most questions involve ticking the most appropriate response, or writing some simple notes. Please feel free to write additional comments in the spaces provided. If you would like assistance with any part of the questionnaire, please ask one of the research team.

Thank you again for taking the time to fill in this questionnaire.

FAMILY DETAILS

PERSON COMPLETING THIS FORM

1	What is your relationship to the child in our study? (please tick one)	
	Biological mother	
	Biological father	
	Step mother	
	Step father	
	Foster parent	
	Family relation (e.g. aunt, grandmother)	
	Other, please specify:	

2	a) Are you the primary caregiver?	Yes	No
	b) If no, who is the child's primary caregiver? (please tick one)		
	Biological mother		
	Biological father		
	Step mother		
	Step father		
	Foster parent		
	Family relation (e.g. aunt, grandmother)		
	Other, please specify:		

3	Family Living Situation (please tick one)	
	Child living with mother & father	
	Child living with mother only	
	Child living with father only	
	Parents separated/divorced but both have custody rights	
	Other, please specify:	

4	How many brothers and sisters does your child have?	

5	How many children live at home?	

6	How many adults (older than 21 years) live at home?	

7	Language spoken at home (please circle one)	
	Only language spoken at home is English	
	English is one of the languages spoken at home	
	Little or no English spoken at home	
	Please indicate which is the other main language spoken at home (if not English)…… ……	

Child's Mother or Primary Caregiver

8	How old were you at your last birthday?	Years	

9 What ethnic groups do you belong to or identify with? (e.g. Australian, Aboriginal, Italian, Greek)

1.
2.
3.

10 Which of the following best describes your highest level of education? (please circle one)

Left school between 13-16 years, no formal qualifications	
Completed Year 11	
High School Certificate (completed Yr 12)	
Professional qualifications without a degree	
University degree	
Post graduate degree	

11	a) Do you have other qualifications (e.g. trade, secretarial)	Yes	No

b) If yes, please specify

……
…….

12 a) Are you working at the moment?

Yes, full time	
Yes, part time (< 30hours)	
No.	

b) If yes, please provide a title & description of the job.

_____
_____
_____
_____

13 Are you currently living with a partner?

Yes, legally married	
Yes, defacto relationship	
No	

If you answered "No", please go to section 19.

Primary Caregiver's Partner

14	How old was your partner at his/her last birthday?	Years	

15 What ethnic groups does your partner belong to or identify with? (e.g. Australian, Aboriginal Italian, Greek)

1.
2.
3.

16 Which of the following best describes your partner's highest level of education? (please circle one)

Left school between 13-16 years, no formal qualifications	
Completed Year 11	
High School Certificate (completed Year 12)	
Professional qualifications without a degree	
University degree	
Post graduate degree	
Don't know	

17	a) Does your partner have other qualifications (e.g. trade, secretarial)	Yes	No

b) If yes, please specify

……
……
……
……

18 a) Is the partner working at the moment?

Yes, full time	
Yes, part time (< 30hours)	
No.	

b) If yes, please provide a title & description of the job

_____
_____
_____
_____

19	Any other comments?	Yes	No

b) If yes, please specify

……
……
……
……

Thank you for completing this questionnaire

Parameters of the proposed imaging sequences:

Sequence Parameters	T2 map (3 echo)	T1-MPRAGE	Field Map	DWI-30 (b=1000s/mm2)	DWI-65 (b=2000s/mm2)	ASL	T1-TSE2	T2-Haste (Axial)	T2-Haste (Cor)	T2-Haste (Sag)
Repetition time (ms)	10580	2100	488	9500	9500	3427.5	1490	2000	2000	2000
Echo time (ms)	27, 122, 189	3.18	4.9, 7.4	130	130	21	12	90	101	88
Flip Angle	150	9	60	90	90	90	147	150	150	150
Field of view (mm)	180	160	160	224	224	256	180	180	180	200
FoV Phase	79.70%	100%	100%	100%	100%	100%	70%	70%	70%	80%
Matrix	256 x 204	128 x 128	64 x 64	128 x 128	128 x 128	64 x 64	256 x 180	320 x 224	320 x 224	320 x 256
Voxel size (mm)	0.7 x 0.7	1.25x1.25	2.5 x 2.5	1.75 x 1.75	1.75 x 1.75	4 x 4	0.7 x 0.7	0.56 x 0.56	0.56 x 0.56	0.625 x 0.625
Dist. Factor	0%		25%	0%	0%	10%	0%	20%	20%	20%
Slice thickness (mm)	2.0	1.3	2.6	2.0	2.0	5.0	2.0	4.0	4.0	4.0
Number of slices	47	96	29	47	47	17	47	21	21	23
Fat Suppression	None	None	None	Fat sat (weak)	Fat sat (weak)	Fat Sat (Strong)	None	None	None	None
Phase partial fourier	Off	Off	Off	6/8	6/8	7/8	Off	5/8	5/8	5/8
Grappa (accel/RefLines)	None	"2/24"	None	"2/24"	"2/24"	None	None	"2/24"	"2/24"	"2/24"
Bandwidth (Hz/Px)	130	180	260	1400	1400	2230	260	400	400	400
Echo spacing (ms)	13.5	7.7		0.82	0.82	0.51	11.5	5.2	5.2	5.2
Turbo (echo trains)/EPI Factor	5 (31)			128	128	64	2 (68)	179	179	205
Examination time (m:s)	5:20	3:00	1:05	5:25	10:48	5:17	1:44	0:44	0:44	0:48

Flow compensated.

* ASL perfusion parameters are Inversion time (1/2) = 700 and 1800 ms. Saturation stop time 1600 ms.

PPREMO Study (Prediction of PREterm Motor Outcomes)

Paediatrician Assessment (Version 1; 30/5/14)

Study ID: **Date:**

Child's name			
Mother's name			
Fathers name			
EDD			
Corrected Age			
Weight	kg / percentile		
Height	cm / percentile		
Head Circumference	cm / percentile		
Method of nutrition	Not assessed =0		Comments
	Full Oral Feeds =1		
	Modified textures = 2		
	Tube- nasogastric =3		
	PEG = 4		
Visual impairment (after correction, on the better eye)	Not assessed =0		
	Normal/No visual impairment =1		
	Squint =2		
	Impaired =3		
	Severely impaired (blind or no useful vision) =4		
Hearing impairment (before correction, on the better ear)	Not assessed =0		
	Normal =1		
	Impaired =2		
	Severely impaired (hearing loss > 70 dB) =3		
General Observation:	No abnormality =0	Abnormality=1	
Face	0	1	
dysmorphism	0	1	
general nutritional state	0	1	
Body proportions	0	1	
Muscle bulk	0	1	
symmetry	0	1	
tongue fasciculation	0	1	
excessive drooling	0	1	
other	0	1	
Motor development:	Yes=0	No=1	
Rolling independently	0	1	
Sitting independently	0	1	
Crawling indep.	0	1	
Pull-to-stand indep.	0	1	
Standing independently	0	1	
Walking independently	0	1	
Gait:	Not assessed = 0		
	Age appropriate = 1		
	Toe walking = 2		
	Asymmetrical gait = 3		

Neurological Signs:

Tone:	Left				Right			
Upper Limbs	Not tested = 0	Normal =1	Hypotonic =2	Hypertonic =3	Not tested = 0	Normal =1	Hypotonic =2	Hypertonic =3
Lower limbs	Not tested = 0	Normal =1	Hypotonic =2	Hypertonic =3	Not tested = 0	Normal =1	Hypotonic =2	Hypertonic =3

Strength:												
Upper Limbs	Not tested = 0	1	2	3	4	5	Not tested = 0	1	2	3	4	5
Lower limbs	Not tested = 0	1	2	3	4	5	Not tested = 0	1	2	3	4	5

Tendon Reflexes:	Left	Right
Upper Limbs	Not tested =0 Present/Normal =1 Absent =2 Depressed =3 Brisk =4 Hyperreflexic/Very Brisk =5	Not tested =0 Present/Normal =1 Absent =2 Depressed =3 Brisk =4 Hyperreflexic/Very Brisk =5
Lower limbs	Not tested =0 Present/Normal =1 Absent =2 Depressed =3 Brisk =4 Hyperreflexic/Very Brisk =5	Not tested =0 Present/Normal =1 Absent =2 Depressed =3 Brisk =4 Hyperreflexic/Very Brisk =5

Clonus:						
Upper Limbs	Not tested = 0	Absent =1	Present =2	Not tested = 0	Absent =1	Present =2
Lower limbs	Not tested = 0	Absent =1	Present =2	Not tested = 0	Absent =1	Present =2

Plantar reflexes:							
Not tested = 0	Normal ↓ =1	No response =2	Abnormal ↑=3	Not tested = 0	Normal ↓ =1	No response =2	Abnormal ↑=3

Cranial Nerves	Not tested = 0, Normal = 1, Abnormal = 2			Comments
II	0	1	2	
III, IV, VI	0	1	2	
V	0	1	2	
VII	0	1	2	
VIII	0	1	2	
IX, X, XII	0	1	2	
XI	0	1	2	

Summary:

Neurological Status	Normal = 0	Unspecified signs = 1	Abnormal (signs of CP) = 2
Cerebral palsy	No =0	Possibly =1	Definitely =2
Comments			

Patterns of motor impairment

Motor type	**Primary**	**Secondary**
	Spastic =1	Spastic =1
	dyskinetic- dystonic =2	dyskinetic- dystonic =2
	dyskinetic- choreoathetotic =3	dyskinetic- choreoathetotic =3
	Hypotonic =4	Hypotonic =4
	Ataxic =5	Ataxic =5
Distribution	Bilateral =1 / unilateral =2	Bilateral =1 / unilateral =2
	No of limbs 1 / 2 / 3 / 4	No of limbs 1 / 2 / 3 / 4

Functional level

Gross Motor Function Classification System for Cerebral Palsy (GMFCS) Before 2nd Birthday

Level I Infants move in and out of sitting and floor sit with both hands free to manipulate objects. Infants crawl on hands and knees, pull to stand and take steps holding on to furniture. Infants walk between 18 months and 2 years of age without the need for any assistive mobility device.

Level II Infants maintain floor sitting but may need to use their hands for support to maintain balance. Infants creep on their stomach or crawl on hands and knees. Infants may pull to stand and take steps holding on to furniture.

Level III Infants maintain floor sitting when the low back is supported. Infants roll and creep forward on their stomachs.

Level IV Infants have head control but trunk support is required for floor sitting. Infants can roll to supine and may roll to prone.

Level V Physical impairments limit voluntary control of movement. Infants are unable to maintain antigravity head and trunk postures in prone and sitting. Infants require adult assistance to roll.

GMFCS level (0-2 years scale)	I =1 / II =2 / III =3 / IV= 4 / V= 5	
Upper limb/ Handedness	Right predominant =0 Left predominant =1 Bilateral =2	

Recommendations:

Suggest referral to GP for further management	
Suggest referral to General Paediatrician for further management	
Suggest referral to Community CDS for further assessment	
Suggest referral to Neuropaediatric clinic at RCH for further assessment	
Other:	

Classification and Definition of Disorders Causing Hypertonia in Childhood

References: *Sanger et al (2003) Pediatrics 111(1), e89-e97*[1]
SCPE (2000) Dev Med and Child Neuro 42: 816-824[2]

Spasticity[1] is defined as hypertonia in which 1 or both of the following signs are present:

1) resistance to externally imposed movement increases with increasing speed of stretch and varies with the direction of joint movement, and/or
2) resistance to externally imposed movement rises rapidly above a threshold speed or joint angle.

Dystonia[1] is defined as a movement disorder in which involuntary sustained or intermittent muscle contractions cause twisting and repetitive movements, abnormal postures, or both. Chorea means rapid involuntary, jerky often fragmented movements. Athetosis means slower, constantly changing, writhing or contorting movements[2]

Ataxia[2] is characterized by:

- Loss of orderly muscular coordination so that movements are performed with abnormal force, rhythm and accuracy.
- Typical features are: trunk and gait ataxia- disturbed balance, past-pointing- over- or undershooting of goal-directed movements

Rigidity [1] is defined as hypertonia in which all of the following are true:

1) the resistance to externally imposed joint movement is present at very low speeds of movement, does not depend on imposed speed, and does not exhibit a speed or angle threshold;
2) simultaneous co-contraction of agonists and antagonists may occur, and this is reflected in an immediate resistance to a reversal of the direction of movement about a joint;
3) the limb does not tend to return toward a particular fixed posture or extreme joint angle; and
4) voluntary activity in distant muscle groups does not lead to involuntary movements about the rigid joints, although rigidity may worsen.