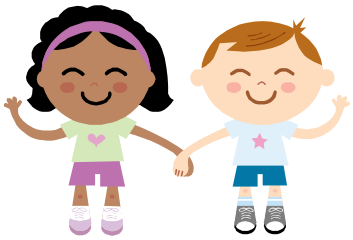
CHYLD



Children with Hypoglycaemia and their Later Development

Mid-childhood outcomes of children born at risk of neonatal hypoglycaemia

STUDY PROTOCOL

ADMINISTRATIVE INFORMATION

Title: Mid-childhood outcomes of children born at risk of neonatal hypoglycaemia; CHYLD Study

Short title: CHYLD mid-childhood outcomes

Registration: Sugar Babies (ANZCTR2608000623392)

Protocol details: version 1.1, dated 17.11.2016

Funding: Health Research Council New Zealand, Maurice and Phyllis Paykel Trust

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1 INTRODUCTION

1.1 Background and Rationale

Hypoglycaemia is the most frequent metabolic disorder of the newborn, affecting up to 15% of all babies,[1] and the only known common preventable cause of brain damage in infancy. Even transient asymptomatic neonatal hypoglycaemia may contribute to educational under-achievement.[2] However, evidence to guide management of this common condition is limited as there have been no large prospective studies in infants born at risk of hypoglycaemia that have assessed school-age outcomes.

Recent recommendations to increase blood glucose thresholds for diagnosis and treatment of neonatal hypoglycaemia[3] to avoid hypoglycaemic brain injury may be ill advised, as there is potential for iatrogenic harm with additional intervention. For example, increased use of formula and admission of babies to neonatal intensive care (NICU) is associated with lower breastfeeding rates[4] and pain-induced stress from heel lancing has been associated with impaired cortical maturation.[5] We have also shown that babies with higher glucose concentrations after hypoglycaemia are at increased risk of neurodevelopmental delay,[6] suggesting that rapid correction of hypoglycaemia may have adverse effects on brain development, a finding that has also been demonstrated in animals.[7] Thus the best approach to the diagnosis and management of neonatal hypoglycaemia remains unclear.[8]

To address this knowledge gap we established a prospective cohort, the CHYLD Study (Children born at risk of neonatal hypoglycaemia), which recruited 614 term and moderate-late preterm babies born at risk of neonatal hypoglycaemia in a single centre and managed according to standard clinical guidelines, including regular blood glucose monitoring using a gold standard glucose oxidase method, with the aim of maintaining blood glucose concentrations ≥2.6 mM. Half of these babies developed clinical hypoglycaemia.[6] A major strength of this study is that two thirds of the cohort underwent masked continuous interstitial glucose monitoring, providing detailed information about neonatal glycaemia, including clinically undetected low glucose concentrations.[9] Thus the cohort includes children who never experienced neonatal hypoglycaemia, children whose hypoglycaemia was detected and treated, and children whose low glucose concentrations were not detected clinically (interstitial monitor only) and therefore were untreated.

Children in the CHYLD cohort underwent in-depth assessment at 2 and 4.5 years of age with standardised measures of growth, neurological function, vision, cognitive and language development, memory, executive function, general health and family environment. At 2 years, 37% had some evidence of neurosensory impairment, but we found no relationship between the occurrence, severity or frequency of neonatal low glucose concentrations and developmental outcome.[6] Intriguingly, high glucose concentrations and less glucose stability in the first 48 hours was associated with relatively large increases (40-70%) in the risk of impairment, particularly cognitive delay.[6]

At 4.5 years, neurosensory impairment remained common (38%), and again we detected no relationship between cognitive function and neonatal hypoglycaemia.[10] However, hypoglycaemia was associated with a 2-3-fold increased risk of poor executive function and visual-motor integration at this age, with greater impairment in children with severe or recurrent hypoglycaemia. Of particular concern was a 4-fold increase in the risk of executive function difficulty in children who had experienced undetected and thus untreated low neonatal glucose concentrations (interstitial monitor only).

These findings suggest that neonatal hypoglycaemia is associated with subtle impairment in specific skills that only become apparent at later ages, and that current operational thresholds for diagnosis and treatment of neonatal hypoglycaemia may need to be revised. Executive function and visual-motor integration are skills that are affected by other perinatal insults, such as preterm birth, and are important for successful learning and school performance.[11] It will therefore be important to determine whether the poorer performance in

these skills at 4.5 years has functional significance in terms of difficulties at school age. Furthermore, magnetic resonance imaging (MRI) of the brain is feasible in school age children.[12] MRI can quantify the impact of neonatal hypoglycaemia on brain structure and function in mid-childhood and provide a link between behavioural outcomes and brain development.

Therefore, we propose a further follow-up study of the CHYLD cohort at 9-10 years of age focusing on neurocognitive processing and educational achievement, together with evidence of structural changes in the brain on neuroimaging.

1.2 Objectives and Hypothesis

The objectives of the CHYLD Mid-Childhood Outcomes study are to determine at 9-10 years of age the effect of neonatal hypoglycaemia, including frequency, severity, and glycaemic response to treatment, on:

i. Neurocognitive function:
    a. Executive function (attention, memory and inhibition)
    b. Visual perception
    c. Fine motor skills
    d. Visual-motor integration
    e. Academic achievement (reading, mathematics, writing)
    f. Psychosocial adaptation (social, emotional, behavioural)
ii. Brain structure, including volume and tractography.

The study hypothesis is that neurocognitive function and brain structure is related to the frequency and severity of low glucose concentrations and to glycaemic instability in the neonatal period.

1.3 Study Design

CHYLD is a prospective longitudinal study of children born from 32 weeks' gestation with one or more risk factors for neonatal hypoglycaemia. The present study will aim to assess all children remaining in the cohort at 9-10 years of age.

1.4 Neonatal Studies

The CHYLD Study includes children recruited to the BABIES[9] and Sugar Babies[4] study and born at Waikato Women's Hospital, Hamilton, New Zealand. Newborn babies were eligible to enter these studies after being identified as having a risk of, or requiring treatment for, neonatal hypoglycaemia.

The aim of the BABIES study was to investigate the relationship between blood glucose concentrations, alternative cerebral fuels and brain function in newborn babies at risk of hypoglycaemia. Babies recruited to this study were of 32 weeks' gestation or more and admitted to the Neonatal Intensive Care Unit (NICU). In addition to EEG monitoring, and continuous glucose monitoring, these babies had up to 4 blood samples taken for the measurement of alternative cerebral fuels. A total of 102 babies were recruited between December 2006 and February 2009.

The Sugar Babies study was a randomised trial to determine the effectiveness of treatment with 40% dextrose gel in reversing neonatal hypoglycaemia in the first 48 hours after birth. Babies recruited to this study were 35 weeks' gestation or greater and less than 48 hours old. Babies who became hypoglycaemic were randomised to treatment with dextrose gel or placebo. The primary outcome of the trial was treatment failure, defined as a blood glucose concentration of <2.6mM 30 min after the second of two treatment episodes. Recruitment to the Sugar Babies study began in November 2008 and concluded in December 2010, with a total of 514 babies recruited.

For both studies, babies identified as being at risk of hypoglycaemia included:

- Small babies (birth weight <10th percentile or <2500g)
- Large babies (birth weight >90th percentile or >4500g)
- Babies of diabetic mothers
- Babies who are stressed i.e. those suffering from sepsis, haemolytic disease of the newborn, respiratory distress, congenital heart disease, or not feeding well

Most of the babies were recruited prior to birth; however, some eligible babies were entered into the studies after birth. All babies were managed according to the clinical guidelines of the Newborn Intensive Care Unit at Waikato hospital. For well babies, early skin-to-skin contact and demand breast feeding was encouraged, and babies roomed in with their mothers. For babies admitted to NICU, fluids were provided at 60, 90, 120, 150 ml/kg on days one to four respectively. Early oral feeds were commenced with breast milk as soon as it was available. Infant formula was given with parental permission until adequate breast milk was available. If the baby was unable to tolerate feeds, intravenous 10% dextrose was given and gradually reduced as oral feeds were tolerated.

Hypoglycaemia was defined as a blood glucose concentration <2.6mmol/l. Initial management for a hypoglycaemic well baby was 40% dextrose gel (200 mg/kg) (or placebo, for babies randomised to the placebo arm of the Sugar Babies study) massaged into the buccal membranes and milk at 7.5 ml/kg (90 ml/kg/day) given two hourly. If the hypoglycaemia did not resolve, the dextrose gel was repeated once. Additional measures could include continuous milk and adding Polycose (4g/100ml) to the feed. In an unwell baby or a baby not responding to these measures, intravenous 10% dextrose (2 ml/kg) was given over ten minutes followed by intravenous dextrose at 60 or 90 ml/kg/day (4 - 6 mg/kg/min). If the intravenous dextrose rate increased beyond 9 mg/kg/min additional investigations were initiated.

Blood glucose concentration was measured by heel-prick sampling at one hour of age, then before feeds two to four hourly for 12 hours. In babies receiving intravenous dextrose, blood glucose concentrations were measured 4 hourly for 12 hours, and then less frequently as clinically indicated. In babies receiving treatment for hypoglycaemia, blood glucose concentrations were measured again thirty minutes after treatment. All blood glucose concentrations were measured on a blood gas analyser (Radiometer, ABL800Flex, Copenhagen) using the glucose oxidase method (reading range 0.0 to 60 mmol/l, coefficient of variation 2.1%).

In all babies participating in these studies whose parents gave consent, a continuous glucose monitor sensor (CGMS® system gold ™ Medtronic, MiniMed, Northridge,CA, USA) was inserted into the lateral aspect of the thigh as soon as possible after recruitment, and remained in place for at least 48 h and up to 7 d. The data collected from the CGMS were downloaded at the end of the study period, and were therefore not available to clinicians and could not influence the clinical management of hypoglycaemia in these babies.

Although none of these children developed symptoms of hypoglycaemia, approximately half were identified by routine blood tests as having developed hypoglycaemia and a further 20-30% with untreated asymptomatic hypoglycaemia were identified via CGMS. This means, therefore, that some of these babies experienced asymptomatic hypoglycaemia for which they were not treated.

1.5 Two-year and Four-and-a-half-year Follow-up Studies

In the two-year follow-up study, children born from 35 weeks' gestation were assessed at 24 months' corrected age ± 1 month.[6] Those born before 35 weeks' were excluded from this study due restrictions on funding and other logistical reasons. However, all children were eligible for the four-and-a-half-year follow-up study, including those born from 32 to 35 weeks'. This study was conducted at 54 months' corrected age

± 2 months. Children who had suffered significant brain injury or neurological illness were excluded from data analysis.

In both previous studies, children underwent a comprehensive assessment of neurodevelopment, vision, general health and assessment of socio-demographic characteristics of the family at both 2 and 4.5 years (Table 1).

Table 1 Summary of 2 and 4.5 year assessments

Developmental Domain	Measure	Skills or Function	2 Yrs	4.5 Yrs
Cognitive	Bayley-III[13]	Play, attention, memory, problem solving	x	
	WPPSI-III	Verbal, performance (fluid), processing speed, general language & full scale IQ		x
Language	Bayley-III[13]	Receptive, expressive language	x	
	WPPSI-III	Language component		x
Motor and Visual Perception	Bayley-III[13]	Fine and gross motor skills	x	
	Beery-Buktenica Developmental Test of VMI, 6th Edition	Integration of visual and motor skills, visual perception and coordination		x
Auditory Processing	Phelps Kindergarten Readiness Scale (Items 6-8)	Auditory discrimination, auditory memory		x
Social-Emotional and Adaptive Behaviour	Bayley-III[13] (PQ)	Pretend play, sensory processes, communication of basic wishes	x	
	Bayley-III (PQ)	Attainment of functional skills	x	
	Strengths and Difficulties Questionnaire (PQ)	Emotional symptoms, conduct problems, hyperactivity/inattention, peer relationships, pro-social behaviour		x
	Child Behaviour Checklist (PQ)	Behaviour Home & School		x
	Social Communication Questionnaire (Lifetime form) (PQ)	Autistic spectrum disorder traits		x
Executive Function	BRIEF-P (PQ)	Parent report of executive function	x	x
	Multi-search/Multi-location[14]	Simple working memory – holding information in mind over delay and response shifting	x	
	Snack Delay[15]	Simple response inhibition - withholding/delay prepotent or automatic response	x	
	Fruit Stroop[15]	Complex response inhibition - holding rule in mind, responding according to rule & inhibiting a prepotent response	x	
	Reverse categorization	Complex response inhibition	x	
	Gift Wrap Delay	Simple response inhibition		x
	Bear/Dragon	Complex response inhibition		x
	Dimensional Change Card Sort (DCCS)	Attention shifting		x
	Day/Night (Stroop)	Complex response inhibition - holding rule in mind, responding according to rule & inhibiting a prepotent response		x
Vision	External observation	External ocular health	x	x

Developmental Domain	Measure	Skills or Function	2 Yrs	4.5 Yrs
	Bruckner's/red reflexes, pupil examination	Internal ocular health	x	x
	Direct ophthalmoscopy	Ocular health		x
	Teller Acuity/Cardiff Cards	Visual acuity	x	
	Letter matching, picture matching/naming	Visual acuity		x
	Sheridan Gardiner Test	Visual Acuity		x
	Cover test, Hirschberg reflexes	Ocular alignment	x	x
	Static distance retinoscopy, Autorefraction (SureSight)	Non-cycloplegic Refraction	x	x
	Frisby plates, Lang I and II	Stereopsis	x	
	TNO, Randot Children's Test	Stereopsis		x
	20diopter Prism Test	Motor fusion	x	x
	Following soft toy/light	Motility/tracking	x	x
	Motion coherence threshold (global dot motion perception) – detection (OKN response), perception (behavioural response)[16, 17]	Function of the dorsal visual cortical stream	x	x
Neurological	Neurological exam	Tone, reflexes, coordination	x	x
	Movement Battery for Children (Movement ABC-2)[18]	Gross and fine motor skills		x
	Gross Motor Classification System (Palisano)	Gross motor function, severity of cerebral palsy		x
Growth	Standard measures	Height, weight, head circumference, abdominal circumference, Body Mass Index (BMI)	x	x
General Health	General Health (PQ)[19]	Sleeping, breastfeeding/diet/nutrition, illnesses, hospitalisations	x	x
Family & Environment	CHYLD Caregiver questionnaire (PQ)	Socioeconomic status, parent education, health, siblings, household, alcohol, tobacco & other drug use	x	x

PQ = Parent Questionnaire

2 METHODS

2.1 Participants, Study Setting and Outcomes

2.1.1 Eligibility Criteria

All children born at ≥32 weeks' gestation who have not been previously withdrawn from the cohort will be eligible for this Mid-Childhood Outcomes study. Children who have suffered brain injury through a known accident or serious illness will be excluded, but their details will be recorded for completeness.

2.1.2 Contact Tracing

Contact tracing will be performed using the CHYLD cohort database which has the contact details provided by families at 4.5 years. Those who have previously withdrawn will be marked as not eligible, and a check will be made of NHI numbers to ensure we avoid contacting families whose child has died. Two months before the child's 9th birthday, families will be sent a letter, along with the Participant Information Sheet, inviting

them to participate in the follow-up study and asking them to contact the study team. After 1-2 weeks, if we have not heard from the family, a member of research team will attempt to make contact by phone to check if the information has been received and to discuss the study. If we are unable to make contact, we will trace families via alternative contacts in the study contact database or via the primary health provider.

Caregivers will be given sufficient time to consider the study and we will offer to meet with them and their whānau to provide further information and answer questions. All caregivers will be provided with the Participant Information Sheet and informed consent will be sought from caregivers, either over the phone or in person.

2.1.3 Follow-up Location

Where possible children will have their neurocognitive assessment at school, as we have previously found that this is more convenient for parents and facilitates high follow-up rates.[20, 21] Once informed consent has been obtained, the child's school will be contacted and a suitable time organised to undertake the assessment. Caregivers will be invited to attend the school assessment if they wish. The assessment will be conducted in a standardised order with breaks as required

If informed consent is obtained for brain MRI, this will be performed either at Waikato Hospital (Midlands Radiology) or the Centre of Advanced MRI (CAMRI), University of Auckland. The MRI will usually be scheduled separately, but some children attending CAMRI may be able to have their neurocognitive assessment at the Liggins Institute on the same day. Both sites have experience with performing MRI in children.

2.1.4 Assessments

Children will be assessed at 9-10 years' corrected age by suitably trained assessors. This age was chosen so that children will be seen in Years 5 and 6 prior to intermediate school, when educational influences and opportunities begin to change substantially. Children will be assessed as follows (Table 2):

Table 2. Summary of 9-to 10-year old assessments

Domain	Measure	Skills or function	Norms
Executive function	Tablet based battery of tests (CANTAB)	Attention, memory, inhibition	tbc
	BRIEF – (PQ) BRIEF – (TQ)	Parent and teacher report of executive function	Test norms (US)
Motor skills	MABC-2	Fine motor skills	Test norms (UK)
Motor and visual perception and processing	Beery-Buktenica test, 6th edition	Integration of visual and motor skills, visual perception and coordination	Test norms (US)
	Laptop based tests of motion coherence and form coherence	Function of the dorsal visual cortical stream	tbc
Academic achievement	Computer based school achievement tests (e-asTTle)	Reading Comprehension and Mathematics (English Curriculum); Pānui and Pāngarau (Māori medium)	Test norms (NZ)
	Teacher questionnaire	Teacher report of academic performance	Descriptive data
Social-emotional and adaptive behaviour	SDQ-P SDQ-T	Emotional symptoms, conduct problems, hyperactivity/inattention, peer relationships, pro-social behaviour	Test norms (UK)
	ASQ, P	Autistic spectrum disorder traits, parental report	Test norms (UK)
General health	CHQ	Parental report of general health, behaviour, limitation in everyday and school activities	Test norms (US)

Family and Environment	Parent questionnaire	Parental report of demographics, socio-economic status, school and health history	Descriptive data
Structural MRI	Brain sequence: T1, T2, DTI and field map, SWI, TIRM (flair)	Cortical morphometry and white matter tractography	tbc

MABC-2, Movement assessment Battery for Children, 2nd edition. SDQ, The Strengths and Difficulties Questionnaire; P, parent questionnaire; T-teacher questionnaire; ASQ, autism spectrum quotient; CHQ, child health questionnaire; MRI, magnetic brain imaging.

Executive function

Cognitive elements of executive function will be tested using a tablet-based battery (CANTAB), focusing on attention (Attention Switching Task), memory (One-touch Stockings of Cambridge, Spatial Working Memory, Paired Associate Learning) and inhibition (Stop Signal Task).[22]

The Attention Switching Task (AST) assesses the ability of a child to switch their attention between the direction of an arrow that appears on the screen and the location of an arrow on the screen in presence of distracting information that is given during testing. The outcome measure will be median response latency (switching and congruent).

The One Touch Stocking of Cambridge (OTS) task involves resolving three problems to position colored balls in the right sequence on the screen in two, three and four moves. The outcome measures will be the number of problems solved on first choice and median latency to first choice.

The Spatial Working Memory (SWM) task involves finding a blue token on the screen by eliminating boxes that also appear on the screen. Two outcome measures will be assessed: between errors (number of times the subject revisits a box in which a token has previously been found, calculated for trials of four, six and eight tokens) and a measure of strategy.

The Paired Associate Learning (PAL) task involves correctly locating patterns that were previously shown on the screen hidden in the boxes. The outcome measures will be total errors (adjusted) and the first attempt memory score (number of correct box choices made on the first attempt during assessment problems).

The Stop Signal Task (SST) involves responding to an arrow stimulus, by touching the right side of the screen where the arrow is pointing. The participant needs to inhibit the response if the audio tone is switched on. The main outcome measure will be the stop signal reaction time (length of time between the go stimulus and the stop stimulus at which the subject is able to successfully inhibit their response on 50% of the trials); two categorical outcomes will also be assessed (failed stop reaction time less than go reaction time, and mean failed stop reaction time increases).

Visual perception

Visual perception will be measured using laptop-based tests of motion coherence and form coherence, which represent global motion processing.[17] Outcome measures will include motion and form coherence thresholds (percentage), and associated z-scores, calculated from previously collected normative data.

Fine motor skills and visual motor integration

Fine motor skills will be assessed using the Movement ABC test, version 2 (MABC-2)[18] and visual-motor integration by the Beery-Buktenica Developmental Test of Visual-Motor Integration, 6th edition (BBVMI-6),[23] both of which were used in the 4.5-year follow-up study.

MABC-2 band 2 tests children from 7 to 10 years. The fine motor subtest will be used, which includes three timed tasks: placing pegs on the peg board, threading a lace and a drawing trail. The outcome measure will be the fine motor component standard score (normative mean 10, SD 3).

BBVMI-6 can be used for ages 2 to 100 years and consistent of 3 tests: fine motor coordination, visual perception and visual-motor integration (VMI). The VMI task involves copy geometric drawings of increasing complexity. The outcome measures will be standard scores for each subtest (normative mean 100, SD 15).

Academic achievement tests

Academic achievement based on a structured teacher questionnaire and the online e-asTTle school achievement tests of Reading Comprehension and Mathematics in English or Māori.[24]

Standardised measures of Reading Comprehension and Mathematics related to the New Zealand Curriculum and Te Marautanga o Aotearoa have been developed at the University of Auckland and deployed to all schools by the Ministry of Education. The e-asTTle tests are designed for students in Years 5 to 10 (with norms for Years 4 to 12 inclusive) and are aligned to Curriculum Levels 2 to 6. Children in Year 5 are expected to have completed Level 2 and should be starting Level 3, though there is much individual variation in rates of progress in the two domains.

For English-medium students, a computer-adaptive version of e-asTTle will be administered starting with a mixture of Level 2 and Level 3 material, but the computer will adjust the difficulty dependent on individual success. In each of the domains of Reading Comprehension and Mathematic, two strands will be tested. Strands were selected to contain objects that are related to executive function and visual-spatial perception (Table 3).

For Māori-medium students a fixed form e-asTTle test will be used with three levels of difficulty (Table 4). The difficulty level will be selected based on the teacher's evaluation of the student's achievement level (Easy for those who are below or well below Year Standard; Medium if at Year Standard; Hard if above Year Standard).

The e-asTTle tests will be limited to 28 minutes per domain, followed by 6 general aptitude questions on interest and self-efficacy, which will take an additional 2 minutes to complete.

Table 3 e-asTTle settings: New Zealand Curriculum (English medium)

Domain	Level	Strands	Time
Reading Comprehension Adaptive	2 and 3 on	Purposes & Audience Structure	28 min
Mathematics Adaptive	2 and 3 on	Position & Orientation Shape	28 min

Table 4 e-asTTle settings: Te Marautanga o Aotearoa (Māori medium)

Domain	Level	Strands	Time
Pānui Most closed questions	Easy: most Level 2, few Level 3 Medium: many Level 2, many Level 3 Hard: few Level 2, most Level 3	Understanding Interpretation & Evaluation	28 min
Pāngarau Most closed questions	Easy: most Level 2, few Level 3 Medium: many Level 2, many Level 3 Hard: few Level 2, most Level 3	Geometry Algebra	28 min

Parent questionnaires

Parents will complete questionnaires about their child's emotional and behavioural development using the Strengths and Difficulties Questionnaire (SDQ),[27] Behavior Rating Inventory of Executive Function (BRIEF),[28] and Autistic Spectrum Quotient questionnaire (AQ-Child).[25,29] In addition, functional health and wellbeing will be assessed using the Child Health Questionnaire (CHQ).[26]

SDQ is a brief questionnaire for 4- to 17-year old children that assesses emotional and behavioural difficulties. The 25 items (questions) are divided into 5 scales, four of which (emotional symptoms, conduct problems, hyperactivity/inattention, peer problems) comprise the Total Difficulties Score (range 0-40). The fifth scale, prosocial behavior, represents the strengths of a child. Borderline and abnormal thresholds have been

defined for the scales and the Total Difficulties Score. We will use the Australian one-sided version for 4- to 10-year old children (without impact score).

The BRIEF questionnaire is used in children aged 5-18 years and assesses behavioural manifestations of executive function. It consists of 86 items in eight non-overlapping clinical scales (inhibit, shift, emotional control, initiate, working memory, plan/organize, organization of materials, monitor). These theoretically and statistically derived scales form two indexes: a) Behavioral Regulation (three scales) and b) Metacognition (five scales), as well as a Global Executive Composite score which takes into account all of the clinical scales and represents the child's overall executive function. There are also two validity scales to measure Negativity and Inconsistency of responses (≥7 indicates a high degree of inconsistency). T-scores are provided for scales, indices and the Global Executive Composite (normative mean 50, SD 10). Scores >65 are considered clinically significant.

AQ-Child is a 50-item questionnaire that assesses autistic traits in children from 4 to 11 years. It has 5 subscales: social skills, attention switching, attention to detail, communication and imagination. If five or fewer answers are missing these can be substituted for the mean item score. A total score >75 (range 0-150) is indicative of an autistic spectrum disorder.

CHQ assesses general health and health-related quality of life, including 14 physical and psychosocial concepts. We will use the parent short form with 28 items. Outcome measures will include physical functioning and psychosocial scaled scores (normative mean 50, SD 10).

Teacher questionnaires

Teachers will complete teacher versions of the SDQ and BRIEF questionnaires, which have similar items and structure to the parent forms. The teacher completing the questionnaire should have known the child for at least one month.

MRI

A subgroup of 100 children born at ≥36 weeks' gestation will be recruited to undergo brain MRI to assess cortical morphometry and white matter tractography (diffuser tensor imaging). Children who had no evidence (blood or interstitial) of neonatal hypoglycaemia (controls) will be compared to those with i) severe (<2.0 mM) or ≥2 episodes of hypoglycaemia; ii) a single mild episode (2.0 to <2.6 mM); and iii) clinically undetected (interstitial only) and thus untreated episodes (25 per group). The high cost of MRI precludes collection of imaging data for the whole cohort. Functional visual imaging is not available at one of the MRI centres and so will not be performed in this study. Scans will take approximately 30 minutes to complete. All films will be screened by a specialist radiologist to exclude clinically important findings. Unexpected findings will be confirmed by a paediatric radiologist, and will be dealt with according to established protocols at each site. Details of the MRI protocol will be provided in Standard Operating Procedures.

2.1.5 Outcomes

Primary outcome

Processing difficulty, defined as impairment in any of: executive function (one or more CANTAB tests), visual-motor integration (BBVMI-6) or visual perception (motion or form coherence). Impairment is defined as performance more than 1 SD below the normative mean.

Secondary outcomes

- Neurocognitive tests: standard scores and proportion of children with impairment
    - CANTAB test: AST, OTS, SWM, PAL, SST
    - BBVMI-6: motor coordination, visual perception, visual-motor integration
    - Visual perception: motion and form coherence thresholds

  - MABC-2 fine motor subtest
- Academic achievement
  - Teacher report of academic achievement in Reading Comprehension, Mathematics and Writing relative to peers (much worse, worse, about the same, better or much better) and National or Year Standard (well below, below, at or above Standard)
  - e-asTTle scaled score for mathematics / Pāngarau (aMs) and reading / Pānui (aRs); e-asTTle curriculum level; academic performance compared to national norms and National or Year Standards (well below, below, at or above Standard)
- Psychosocial questionnaires
  - SDQ-P and SDQ-T Total Difficulties Score and proportion of children with borderline or abnormal result (SDQ-P ≥14 or SDQ-T ≥12); Prosocial social score and proportion of children with borderline or abnormal result (SDQ-P or SDQ-T subscale ≤5)
  - BRIEF-PQ and BRIEF-TQ T-scores for Behavioral Regulation, Metacognition and Global Executive Composite; proportion of children with Global Executive Composite in clinical range (>65)
  - AQ-Child total score and proportion in clinical range (>75)
  - CHQ physical functioning and psychosocial scaled scores
- Brain MRI
  - Corticol morphometry
  - Tractography

2.1.6 Study Timeline

The first 27 children in the cohort will have turned 9 years corrected age by November 2016 and 2 of these children turn 10 years in January 2017. Thus, the study will aim to start recruiting in December, pending ethical approval. We will aim to assess all children at 9 to 10 years' corrected age, which is equivalent to school Years 5 to 6. Data collection is anticipated to be completed in February 2020 when the last children turn 9 years' corrected age.

- October-November 2016: protocol, ethics and HRC grant preparation; pilot assessments and CRFs
- December 2016 to February 2020: build database; assess study participants
- March 2020 to September 2020: Data analysis and publication.

2.1.7 Sample Size

614 children were recruited to the CHYLD Study at birth, of whom ~577 are currently alive and residing in New Zealand. Based on previous data from this cohort we estimate that the incidence of the primary outcome in controls will be 20%. We achieved 78% follow-up at both 2 and 4.5 years and expect to achieve a similar rate in this study (total of 460 children). Thus, we will have 90% power to detect an increase in incidence of the primary outcome from 20% in controls to 33% (RR 1.60) in children exposed to hypoglycaemia (two tailed α=0.05, assuming half are exposed to hypoglycaemia). For continuous outcomes we will be able to detect differences of 0.3 of a standard deviation (SD). The MRI study ANOVA can detect an effect size of 0.38, i.e., differences of 0.95 SD for pairwise comparisons.

2.1.8 Blinding

All assessors will be blinded to the neonatal history of the CHYLD study participants. Neonatal data will be available to assessors after the assessment is completed if required for clinical purposes, .e.g., for clinical referrals.

2.2 Data Collection, Management and Analysis

2.2.1 Data Collection Methods

Data will be collected using a combination of online tests (CANTAB), computerised assessments (visual perception, e-asTTle, MRI analysis), and hard copy questionnaires.

2.2.2 Data Management

Study data will be stored and maintained centrally using an ACCESS / SQL database. Data from questionnaires will be entered manually into the study database and electronic data will be imported directly. Questionnaires will be scored in the database. Appropriate range and logic checks will be used to check for data entry errors. Once data queries are resolved, the data will be locked. Investigators will have access to the data only after data lock.

2.2.3 Statistical Methods

Statistical analysis will be performed with SAS (SAS Institute).

Descriptive information will be provided on all study data collected from the total cohort, at birth and 9-10 years corrected age. Continuous variables will be summarised as numbers of observed and missing values, and mean (standard deviation) or median (inter-quartile range). Categorical variables will be described as frequencies and percentages. Where appropriate, the coded data reviewed by the Steering and Management Committee will be summarised and used in final analysis.

Primary and secondary outcomes will be compared between children who did and did not develop neonatal hypoglycaemia, adjusting for sex, primary risk factor (IDM, preterm, small, large and other) for neonatal hypoglycaemia and deprivation index (NZDPI, ordinal scale 1-10).[32] Achievement scores will be additionally adjusted for duration of schooling.

Exposure effects will be presented as risk ratio or mean difference with 95% confidence intervals. Secondary analyses will relate outcomes to severity, frequency and duration of neonatal hypoglycaemia, including on continuous interstitial glucose monitoring. Outcomes will also be related to glucose stability and glycaemic responses to treatment.[6]

Statistical tests will be two-sided with a significance level of 5%. No adjustment will be made for multiple comparisons on secondary outcomes, of which results will be considered exploratory and interpreted with caution.

Missing data is unlikely to be missing at random. Therefore, we do not propose to perform any imputation of missing outcome data in the primary analysis.

3 ETHICS AND DISSEMINATION

3.1 Research Ethics Approval

Ethical approval will be sought from the Health and Disability Ethics Committee.

3.2 Locality Approval

Locality approval will be sought from the Liggins Institute and Waikato District Health Board.

3.3 Protocol Amendments

All amendments to the final version of this protocol will require review and approval of the Steering Committee, and will be submitted to HDEC and DHB Research Offices, as appropriate. All amendments, including approval date, will be recorded with this protocol.

3.4 Consent

Parents of children who completed 2- and 4.5-year assessments were informed that there would be ongoing follow-up at school age, pending funding. Parents who have expressed a previous wish to withdraw from the study will not be contacted again. Parents / caregivers will be provided with the Participant Information Sheet, and will be given the opportunity to discuss the study and ask questions of research staff. Processes used to ensure confidentiality will be explained. Following this, written informed consent will be obtained on behalf of each participant.

3.5 Confidentiality

Electronic databases will be stored on secure servers and access will be controlled by unique user ID and password. CRF/eCRFs will be identifiable only by study ID, first and last initial, DOB and EDD. Forms will not contain identifiable information such as names, address, or NHI. Extracted data files will contain DOB and EDD, as these are necessary for analysis, but participant initials will be removed. Contact and personal information will be stored separately from CRF/eCRF data. Hard copy questionnaires will be stored in a locked cabinet. They will also be scanned and added to electronic archive. Study reports will contain only summary data and individual participant data will not be reported. Identifiable data will not be released to any third party. Research staff will be certified in good clinical practice.

At the completion of the study, all electronic data will be permanently digitally archived and accessible only to the study investigators. Hard copy records will be stored in a locked cabinet in a secure office, and will be accessible only to the study investigators. Records will be retained for 10 years after the age of majority.

3.6 Feedback of Findings

A short summary of the findings will be sent to the parents. If concerns exist about a child's health, referral to an appropriate health agency, usually the child's primary health provider, will be made with the consent of the parents. Parents, caregivers and their health professionals will have the opportunity to request more detailed report of specific assessment results if required.

Schools will be sent a copy of the relevant educational findings, with the consent of the parent or caregiver.

3.7 Withdrawal

Parents and caregivers will retain the right to withdraw their child from the study at any stage without the need to provide a reason.

3.8 Declaration of Interests

Investigators will declare any financial, intellectual or other potential conflicts of interest, as outlined by the ICMJE, to the Steering Committee.[33] The Steering Committee will decide on how any conflicts of interest are to be managed.

3.9 Access to Data

The Steering Committee and Management Committee will have access to the full dataset and oversee analysis, interpretation and reporting of results. Approval will be sought from the CHYLD Study Steering Committee prior to publication of study data. Care will be taken to avoid duplication in reporting of results.

3.10 Maori consultation

The CHYLD cohort includes a large number of Māori children (30%), thus ensuring applicability of results to Māori. We consulted with the DVC Māori of the University of Auckland, Mr Jim Peters, when initiating the CHYLD Study and will consult further about follow-up. We have engaged with local groups to acknowledge the contribution of Waikato Māori to this study and disseminate findings, including Te Puna Oranga Māori Health Services, Te Kaunihera Kaumatua and members of the Iwi Māori Council.

Our team includes a Māori investigator (Dr Peter Keegan, Waikato-Maniapoto, Ngati Porou) who is an expert in Māori-medium education and Māori student achievement, and a Māori follow-up coordinator (Ms Jenny Rogers, Ngai Tahu) whose community links are essential in achieving high follow-up rates. Ms Rogers will make the initial contact with Māori whānau where appropriate.

4 STUDY MANAGEMENT

4.1 Steering Committee

The Steering Committee will take overall responsibility for all aspects of the study, meeting 4 times a year. Matters arising between meetings may be dealt with by email. The Principal Investigator and study coordinator will be responsible for maintaining a record of correspondence and minutes of meetings.

The Steering Committee comprises:

Professor Jane Harding, Liggins Institute, University of Auckland
Dr Jane Alsweiler, Paediatrics: Child and Youth Health, University of Auckland
Professor Gavin Brown, Learning, Development and Professional Practice, University of Auckland
Professor Geoffrey Chase, Mechanical Engineering, University of Canterbury
Dr Deborah Harris, Newborn Intensive Care Unit Waikato District Health Board
Dr Christopher McKinlay, Liggins Institute, University of Auckland
Associate Professor Benjamin Thompson, Optometry and Vision Science, University of Auckland
Associated Professor Trecia Wouldes, Psychological Medicine, University of Auckland

4.2 Management Committee

A Study Coordinator will be appointed to oversee day-to-day running of the study. They will be supported by a Management Committee, comprising:

Professor Jane Harding, Liggins Institute, University of Auckland
Dr Jane Alsweiler, Paediatrics: Child and Youth Health, University of Auckland
Professor Gavin Brown, Learning, Development and Professional Practice, University of Auckland
Mr Gregory Gamble, Auckland Uniservices, University of Auckland
Dr Peter Keegan, Te Puna Wānanga, University of Auckland
Dr Christopher McKinlay, Liggins Institute, University of Auckland
Associate Professor Benjamin Thompson, Optometry and Vision Science, University of Auckland
Associated Professor Trecia Wouldes, Psychological Medicine, University of Auckland

4.3 Funding

Funding is being sought.

5 APPENDICES

5.1 Participant Information Sheet and Consent

The following documents are to accompany this protocol:

Title	Version	Date
Parent Information Form	1.0	17.10.2016
Child Information Form	1.0	16.10.2016
Consent Form	1.0	16.10.2016

5.2 Case Report Forms

The following case report forms (CRF) are to accompany this protocol:

Title	Version	Date
Parent or Guardian Questionnaire	1.0	18.10.2016
CHYLD Study Teacher Report Form	1.0	17.10.2016
The Strengths and Difficulties Questionnaire – Parent and Teacher	1.0	18.10.2016
BRIEF Parent Questionnaire	1.0	18.10.2016
BRIEF Teacher Questionnaire	1.0	18.10.2016
Autism Spectrum Quotient	1.0	18.10.2016
Child Health Questionnaire –PQ28	1.0	18.10.2016

5.3 Ethical and Locality Approval

The following letters of approval are to accompany this protocol:

Title	Reference	Date

5.4 Protocol Amendments

Protocol version, Date	Amendment(s)	Date accepted by Steering Group	Date ethics notified (or NA)

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