Genomic Imaging in Neonatal Encephalopathy (GENIE STUDY)

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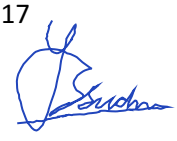
Signature: 

This protocol describes the GENIE Study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator. This study will adhere to the principles outlined in the NHS Research Governance Framework for Health and Social Care (2nd edition). It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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Sponsor:

Imperial College London is the Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

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STUDY SUMMARY

TITLE
Genomic Imaging in Neonatal Encephalopathy

DESIGN
Prospective observational multicentre cohort study

BACKGROUND
Half of the babies receiving cooling therapy for neonatal encephalopathy still have adverse outcomes. Therefore, for the development of personalised neuroprotective therapies it is essential that we are able to rapidly identify those babies who will not respond to cooling, and understand why they do not respond. In this work, we will examine the feasibility of three different genetic classifying methods (host gene expression profile, epigenetics, and copy number variants) for disease stratification in babies with neonatal encephalopathy.

AIMS
1. To compare the host gene expression with brain injury at birth, and neurodisability at two years of age in babies after neonatal encephalopathy
2. To examine the relation of epigenetic changes with brain injury at birth, and neurodisability at two years of age in babies after neonatal encephalopathy
3. To examine the association of copy number variants with brain injury at birth, and neurodisability at two years of age in babies after neonatal encephalopathy

METHODS
A total of 300 term encephalopathic babies will be recruited from participating NHS hospitals over a 3 year period. A small amount of blood will be collected soon after birth and will be send to Imperial College London for genomic analysis. We will also collect blood from the parents of the recruited babies to examine the concordance in their copy number variants. All babies will have magnetic resonance scans performed during the neonatal period and detailed neurodevelopmental assessment between 18 to 24 months, as a part of their routine clinical care.

DATA ANALYSIS AND OUTCOME MEASURES
Next generation sequencing and bioinformatic analysis will be used to identify a minimal set of activated or deactivated genes ('gene signature') that is associated with brain injury patterns and long-term disability.

POPULATION
300 newborn babies with hypoxic ischaemic encephalopathy
600 parents of the recruited babies with hypoxic ischemic encephalopathy.

DURATION
3 year recruitment followed by a neurodevelopmental assessment between 18 to 24 months after birth.

BENEFITS
Our goal is to develop a point-of-care test for disease stratification in neonatal encephalopathy. If successful, this will lead to a paradigm shift in the way we identify and treat babies with this condition, and will open up many new opportunities for the development of individualised neuroprotective therapies.

1. INTRODUCTION

1.1 BACKGROUND

Need for a rapid diagnostic test at birth in neonatal encephalopathy

Neonatal encephalopathy (NE) is presumed to be the end point of an acute and unexpected reduction in blood flow and oxygen to the fetal brain, immediately prior to delivery. A few hours after birth the injury progresses to catastrophic secondary energy failure, leading to permanent brain damage. Therefore, it is vitally important to identify babies at risk of long-term neurodisability as early as possible after birth, to initiate preventative therapy. Without appropriate treatment approximately one third of affected babies die, and up to two thirds develop long-term neurodisability, with a cost to the NHS of £750,000 for each affected child.[1]

In recent years, therapeutic hypothermia (cooling initiated within 6 hours of birth and continued for 3 days) has been adopted in the NHS as the first effective therapy for neonatal encephalopathy. This therapy reduces the likelihood of death (Risk Ratio (RR) 0.75; 95% Confidence Intervals (CI) 0.64 to 0.88), and neurodisability (RR 0.77; 95% CI 0.63 to 0.94) after moderate or severe neonatal encephalopathy, and has led to a substantial reduction in mortality and morbidity, saving the NHS approximately £125 million per year.[2]

Despite cooling, approximately half of these babies still develop adverse outcomes. Although many adjunct neuroprotective therapies have proven efficacy in preclinical models of 'pure hypoxic ischemic injury', clinical translation is challenging due to the heterogeneity of neonatal encephalopathy. In fact, only one third can be attributed to an acute intrapartum hypoxic insult, whilst subacute injury from perinatal infection and inflammation may be responsible for the vast majority.

The successful development of personalised neuroprotection therapies in encephalopathy (for example, in the case of sub-acute brain injury, infection, inflammation or an acute intrapartum event) requires a robust point-of-care test for disease stratification which is based on aetiology, and targeted monitoring of therapeutic pathways. However, although advanced magnetic resonance (MR) biomarkers (whole brain fractional anisotropy, neurite orientation dispersion and density imaging, spectroscopy) are useful in disease stratification, performing MR scans soon after birth is not realistic (i.e. within the therapeutic window period for effective neuroprotection).

Therefore, there is now a concerted effort from the research community to develop *an effective test at birth to identify babies at the highest risk of long-term neurodisability* who can be candidates for additional therapeutic strategies.

Magnetic resonance imaging patterns in neonatal encephalopathy

Magnetic resonance imaging can effectively stratify neonatal encephalopathy based on aetiology. Preclinical and clinical evidence suggest that different MRI patterns of brain injury reflect different aetiologies in neonatal encephalopathy.[3] Sub-acute and chronic hypoxic insults produce a parasagittal watershed zone of infarcts (white matter injury) whereas an acute hypoxic ischaemic event results in injury to the most metabolically active tissues such as the basal ganglia and thalami (BGT), as well as the corticospinal tracts.

Grey matter neurons and early myelinating tissues have a higher metabolic rate than the surrounding white matter. Hence, they are significantly more vulnerable to acute anoxia than the hemispheric white matter, although the duration of ischaemia resulting from a sentinel event may be relatively short. The different MRI injury patterns are related to different outcomes.[3,4] Loss of the normal signal intensity from the motor corticospinal tract is strongly associated with adjacent BGT injury and acts as reliable predictor of abnormal motor outcomes. On the other hand, white matter injury is mainly associated with cognitive function and language development at school age.[5]

Biomarkers for early identification of 'at-risk' encephalopathic infants

The full clinical picture of encephalopathy evolves over the first few days after birth. Hence, clinical examination at birth alone cannot identify all babies at risk of adverse outcome (area under the curve 0.65).[6] Although a number of serum biomarkers have been extensively studied for many years, none have

sufficient accuracy to influence treatment decisions.[7] Early amplitude integrated electroencephalography (aEEG) has a low positive predictive value (60%), which drops even further in cooled infants.[8] Although MR biomarkers are the most accurate predictors of long-term adverse outcomes after neonatal encephalopathy – particularly the lactate:N-acetylaspartate metabolite peak area ratio (Lac/NAA) derived from proton MR spectroscopy (^{1}H MRS) – performing MR scans within few hours after birth is unrealistic.[9] Thus, there is an unmet need for a rapid or point-of-care test for the identification and disease stratification of encephalopathic babies at risk of adverse neurological outcomes, soon after birth.

2. METHODS FOR DISEASE STRATIFICATION

2.1 Gene expression in neonatal encephalopathy

Gene expression profiling (transcriptomics) provides a snapshot of the biological processes that occur in response to internal or external stimuli. Changes in gene activity (expression) occur rapidly, and patterns of increased or decreased transcript abundance can provide both a route map of cellular pathological processes, and a 'signature' used for diagnostic or prognostic classification. Genome wide expression profiling is well established in cancer research for predicting clinical outcome, identifying the presence of metastases, and developing personalised treatment.[10] More recently, specific expression patterns and pathways have been described in several serious infections, including paediatric tuberculosis, allowing disease stratification.[11,12] This is currently being developed as a rapid point-of-care test.

Although gene expression profiling has not yet been reported in babies with neonatal encephalopathy, preclinical data (animal models) suggest up-regulation of genes involved in neurogenesis and autophagy.[13] Furthermore, an overall decrease of endocytotic capacity has been found during hypoxia, suggesting an oxygen-dependent uptake of different macromolecular ligands, or a hypoxia induced paralysis.[14]

2.2 Epigenetics in neonatal encephalopathy

There is growing evidence indicating that epigenetic modification of gene expression, such as DNA methylation or histone modifications, may be one important mechanism underlying the "developmental origins of adult disease".[15] As a heritable epigenetic marker, DNA methylation is the most widely studied epigenetic phenomenon. The aberrant DNA methylation of promoters leads to abnormalities in gene transcription, which ultimately influence gene expression. Much of the epigenome is established during embryogenesis and early development of the fetus. DNA methylation mediates the growth and maintenance of tissue-specific expression profiles in different cell types and plays an essential role in organogenesis and fetal development.[15]

The brain is the central organ responsible for stress responses, and undergoes parallel alterations in its structure and function in response to stress events.[16] There is increasing evidence suggesting that epigenetic machinery orchestrates the development, plasticity, homeostasis and evolutionary innovations of the brain. Recent studies suggest that epigenetic programming in response to fetal hypoxia is responsible for the development of a hypoxic–ischemia sensitive phenotype in the brain, implicating a key role of epigenetic modifications in fetal stress-mediated neuronal and vascular dysfunctions.[17,18]

2.3 Genetic contribution to neonatal encephalopathy

At least a proportion of the encephalopathic babies who develop cerebral palsy can be explained by complex genetics.[19] Copy number variants (CNVs), which are rare (<1%) genetic deletions and duplications, may predispose to cerebral palsy (CP) by interacting with environmental triggers. Predisposing variants are likely to be oligogenic or polygenic and thus additive in nature and require gene-environment investigations. Such variants would increase the risk of a CP phenotype, and not deterministic of CP. Established environmental risk factors for CP, such as IUGR and infection, may interact with predisposing genetic variants and potentiate the chance of a CP outcome.

A recent pilot study has found that ten out of 50 singleton CP cases had potentially relevant CNVs. However all of these were inherited from a healthy parent, suggesting another genetic or environmental contributing factor.[20] Such an inheritance pattern is usual for other neurodevelopmental disorders such as intellectual disability or autism.

In another study from Israel, 16 CP cases out of 52 cases (31%) of unknown aetiology had CNVs that were likely to be pathogenic, and 12 of these were de novo.[21] Currently, the combination of 14% of cases with likely pathogenic point mutations and 20-31% with CNVs of interest gives a potential genetic contribution to causation in up to 34-45% of CP cases.[22]

3. STUDY AIMS

Primary

1. To compare the activity of specific genes in blood (host gene expression) at birth with brain injury on magnetic resonance scans within two weeks of birth, and with neurodisability at two years of age in babies with neonatal encephalopathy

Secondary

1. To identify and characterise the host gene expression pathways associated with basal ganglia/thalamic injury and white matter injury on magnetic resonance scans in neonatal encephalopathy
2. To identify a minimal transcriptomic signature of adverse neurological outcomes
3. To examine the relation of epigenetic changes with brain injury and neurodisability after neonatal encephalopathy
4. To examine the association of copy number variants with brain injury and neurodisability after neonatal encephalopathy

4. PATIENTS AND METHODS

4.1 Study design and sites

The GENIE Study is a prospective observational multicentre cohort study. It is open for all tertiary neonatal units offering cooling therapy to babies with neonatal encephalopathy. The recruiting centres should be offering magnetic resonance scans and neurodevelopmental outcome assessment between 18 to 24 months, as a part of standard clinical care.

4.2 Study participants

We will recruit a total of 300 term or near term (>35 weeks) babies with neonatal encephalopathy over a 3 year period.

4.3 Inclusion criteria

All babies with clinical evidence of encephalopathy (mild, moderate or severe) within 6 hours of birth, along with evidence of an intra-partum asphyxial insult. Whilst most babies may also receive cooling therapy, babies who are not receiving this are also eligible.

Both parents of the encephalopathic babies will be also recruited into the study, and their blood will be collected for testing of copy number variants.

4.4 Exclusion criteria

Babies with life threatening congenital malformations

4.5 Withdrawal criteria

Given the observational nature of this study and the lack of risks to the health of the baby, withdrawal should occur only upon parental request.

4.6 Sample collection and processing

All samples should be collected in pre-assembled sample collection packs containing a PAXgene bottle (for gene expression) and 2 EDTA bottles (for epigenetics and CNVs) with printed barcodes. The sample collection packs will be provided to all recruiting centres.

A total of approximately half a teaspoon (3 ml) of blood will be collected by venepuncture during blood sampling for routine clinical care or via indwelling arterial catheter, into 2 EDTA bottles and 1 PAXgene bottle by local clinicians/nurses, within 6 hours of birth from all recruited babies. In addition, 5ml of blood will be collected by venepuncture from each parent into EDTA bottles by local clinicians/nurses/phlebotomists or research nurses, wherever possible. This will allow us to examine the proportion of CNVs that are genetically transmitted in comparison to de novo mutations. The collected blood samples will be labelled using pre-printed barcodes (anonymised using the study number and date/time of collection) and will be transported to Imperial College London for analysis in batches.

Details of antenatal and postnatal events, and other routine clinical and imaging data will be collected. We will also obtain relevant clinical data from the participants' (baby and mother) medical records or from GPs/local hospitals if needed (GPs will be informed of study participation unless otherwise requested by parents). We will also collect the 3 Tesla MR data that is acquired as a part of routine clinical care. Parents will be contacted regularly via their preferred method of contact. Study newsletters will be sent to parents, unless they opt out.

4.7 Evaluation of neurological outcomes

All babies will have detailed neurodevelopmental evaluation including Bayley Scales of Infant Development (BSID-III or IV if available), Gross Motor Function Classification System (GMFCS), and hearing and vision assessment between 18 to 24 months of age. Severe disability will be defined as any one of the following: both cognitive and language composite BSID-III scores <70, GMFCS level 3–5, hearing impairment requiring hearing aids, or blindness. Moderate disability will be defined as both cognitive and language composite BSID-III scores between 70 and 84 and one or more of the following: GMFCS level 2, hearing impairment with no need for amplification or a persistent seizure disorder. The outcomes will also be assessed by categorisation of these scores and by including mortality as an outcome. An adverse outcome will be defined as death or, in survivors, moderate or severe disability.

5. STATISTICS AND DATA ANALYSIS

Statistical analysis will be performed using R (version 3.3.2). In order to identify lists of differentially expressed transcripts between the patient groups for biological interpretation, we will fit linear regression models and calculate moderated t statistics for each transcript. The p values will be corrected for multiple testing using the Benjamini-Hochberg false discovery rate method to control for type I errors. We will use computational network-based approaches to unravel the underlying biological processes, which produce the gene expression measurements. IPA (http://www.ingenuity.com), DAVID, and a manually curated data set of human gene interactions in InnateDB (http://www.innatedb.com) will be used to examine biological network relationships and any associations with known pathways. To identify signatures of differentially expressed transcripts with diagnostic potential, we will apply variable selection methods to the significantly differentially expressed transcripts using the disease risk score, and its predictive accuracy will be calculated using the pROC package in R. The area under the receiver operating characteristic curve, sensitivity, and specificity will be assessed.

5.1 Sample size calculation

A total sample size of 272 patients (R (v3.3.1) and RNASeqPower (v1.12.0): 108 babies with WM injury, 82 with BGT injury, 82 with normal MR scans and 136 babies with adverse outcome) is required, based on the following assumptions supported by our preliminary data:

(i) RNA-sequencing depth of at least 30 million of reads (ii) Variance of 1.01 between encephalopathic babies with adverse outcomes and those with a normal outcome (iii) Variance of 0.9 between encephalopathic babies with normal MR scans and those with WM injury, and 0.78 between encephalopathic babies with normal MR scans and those with basal ganglia injury (iv) Type I error rate of 0.05 and Type II error rate of 0.1 (90% power). Our preliminary data suggest WM injury in 40%, basal ganglia injury in 30 %, normal MR scans in 30%, and a 50% with an adverse outcome.

We have increased the total sample size to 300 to accommodate a 10% drop-out rate, and poor MR data quality.

6. ADVERSE EVENTS

Given the observational design of this study, there is no anticipated risk of adverse events associated with study participation.

6.1 DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.
Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- Results in death
- Is life-threatening – refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe
- Requires hospitalisation, or prolongation of a current inpatient's hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect

Medical judgement should be exercised in deciding whether an AE is serious in other situations, or performed by a delegated person according to the study delegation log. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation (but may jeopardise the subject or require intervention to prevent one of the other outcomes listed in the definition above), should also be considered serious.

6.2 REPORTING PROCEDURES

All adverse events will be recorded during hospitalisation using the case report form. Babies who suffer hypoxic ischaemic encephalopathy are expected to have higher mortality and morbidity up to two years of age.
All serious adverse events that are not expected to occur in babies with hypoxic ischaemic encephalopathy (systemic venous thrombosis, massive intracranial bleeds), should be reported to the Chief Investigator by completing an SAE form and sending it within 24h. Please send any SAE forms to: p.montaldo@imperial.ac.uk, telephone 0044-20-33132473
The Chief Investigator must notify the Sponsor of all unexpected SAEs. If there was any unexpected SAE which would be considered study related, the Chief Investigator will report to the Ethics Committee within 15 days of becoming aware of the event, using the SAE form. Local investigators should report any SAEs as required by their Sponsor and/or Research & Development Office.

7. REGULATORY ISSUES

7.1 REGULATORY & ETHICS APPROVAL

This study has been reviewed and approved by the Health Research Authority and the South West - Exeter Research Ethics Committee. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions. The study will also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study.

7.2 CONSENT

Since the study aims to obtain information that will inform early treatment decisions (i.e. within 6 hours of birth), it is essential that the data collection begins as soon as possible after birth, and certainly within 6 hours of birth. Hence the blood collection may begin before informed parental consent is obtained (deferred consenting) at the same time as the clinical blood sample collection on admission.

Parents will be informed about the study at the earliest appropriate opportunity (after being explained about their baby's clinical status and when they feel ready) and will be given the Parent Information Leaflet

(PIL). Parents wishing to participate in the study will be asked to sign an informed consent form, once ready, and be given a copy for their records (with the PIL). If the parents do not wish to participate in the study, data collection will be interrupted and the blood samples obtained up to that point will be discarded.

Whether or not the parent(s) decide to take part in the study shall not affect the clinical decisions made during the care of the baby, nor the quality of care provided. All participants are free to withdraw from the study at any time without giving any reason and without prejudicing further treatment.

7.3 CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study according to the Data Protection Act, UK. Personal identification data including telephone numbers and all contact details will be stored (i) As hardcopies in a research folder in locked cupboards in the site Principal Investigator's office, and Imperial College London research office (ii) On NHS computers at the recruiting sites (only for babies recruited from that site) (iii) On a secure and encrypted server at Imperial College London.

All personal data will be stored for a period of 10 years, and will be destroyed using standard Imperial College London protocols (including removal by specialist software for electronic data), unless parental consent for further research is obtained at that time.

Gene expression data will be stored without any identifying information, apart from reference study numbers. All MR data is stripped of identifying information upon export from the scanner, and all other records only reference study numbers. Any remaining potentially sensitive data which are necessary to know for the purposes of study (e.g. date of birth) will be kept on a central, backed-up and encrypted drive on the Imperial College London network, which is only accessible from a separate physical location. Access to each of these areas is tightly controlled, and new users requiring access to these data will require formal authorisation from the Chief Investigator.

MR data will be again anonymised using the study number, and encrypted with a password prior to transfer. Imperial College London file transfer protocols will be used for data transfer. All research data will be stored at Imperial College London, for a period of 10 years.

7.4 INDEMNITY

Imperial College London holds insurance policies for both negligent harm and non-negligent harm, which apply to this study.

7.5 SPONSOR

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

7.6 FUNDING

The Medical Research Council, UK and Garfield Weston Foundation funds this study. There are no payments offered to the study participants.

7.7 AUDITS

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to both GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

8. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through the Centre for Perinatal Neuroscience, Imperial College London. For any queries, please email p.montaldo@imperial.ac.uk.

9. PUBLICATION POLICY

Primary study outcomes will be examined only after the end of the follow-up period. Secondary study outcomes will be examined after the end of the recruitment period (during follow-up time). Study results will be published in relevant peer-reviewed scientific journals as well as in open-access journals as per Imperial College London policy.

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