Study Title:	**The NeuralNET: Research to impact diagnosis, mechanistic understanding and treatment of children's brain and mental health disorders – A pilot study in cerebral palsy**
Short title:	The NeuralNET Cerebral Palsy Pilot Study

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Funder:	Rosetrees Trust, Isaac Newton Trust, and NIHR Cambridge BRC Illumina and NHS East Genomics Laboratory Hub will provide support in kind There are no potential conflicts of interest with the funder.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsors, the Investigator Team, HRA, host organisations, and members of the Research Ethics Committee, unless authorised to do so.

Study Title: The NeuralNET: Research to impact diagnosis, mechanistic understanding and treatment of children's brain and mental health disorders – A pilot study in cerebral palsy

Protocol Date: 22 May 2023

Protocol Version No: 4.0

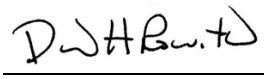
Protocol signature page

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

Chief Investigator:

David H Rowitch		20-10-23	Professor of Paediatrics
(Please print name)	Signature	Date	

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1. LAY SUMMARY

The NeuralNET Cerebral Palsy Pilot Study is testing a genetic testing pathway in the NHS for children with cerebral palsy (CP). Other studies suggest that almost one in three peoples' CP is caused by a change in their genes, but more studies are needed to confirm this. A genetic test called whole genome sequencing (WGS) will be used for children who have CP to look for rare changes in genes that cause the condition, and the results of the test will be given to children's doctors within 12 weeks. Knowing that CP has a genetic cause could lead to changes being made to a child's care or treatment that could improve their condition.

The study will test 66 children with CP from 3 hospitals, and also their biological parent(s), if they're available. Following informed consent, we will collect a blood sample from everyone taking part which will be sent for WGS. It is important to understand what families think and feel about the testing. We will ask parents/guardians of the children taking part to fill in two questionnaires, one before and one after WGS. Some parents/guardians will also be interviewed after getting the WGS result, to ask about their experience of the testing. The study will take up to 16 months per family.

The results of this pilot study will tell us if it is feasible for the NHS to use WGS to test children with CP. If so, a larger study testing more children with CP can then be carried out to help decide if this type of WGS-based testing should be made available through the NHS to children with CP whose clinical care might be changed by the result. The genetic findings from this study will also be made available to other researchers and doctors to do more research into CP that might help us better understand and treat the condition.

2. SYNOPSIS

Study Title	The NeuralNET: Research to impact diagnosis, mechanistic understanding and treatment of children's brain and mental health disorders – a pilot study in cerebral palsy
Short title / acronym	The NeuralNET Cerebral Palsy Pilot Study
Study Design	Mixed methods convergent parallel design
Research questions	Is it feasible to implement a trio whole genome sequencing study to test and diagnose children with cerebral palsy in NHS clinics?
Objectives	1. To measure the uptake of WGS testing by families with a child with CP and to gain insight into families' decisions to decline WGS testing; 2. To identify specific genetic contributors in children with CP recruited to this study; 3. To identify potentially pathogenic variants of unknown significance in children with CP for further investigation of pathogenicity via follow-on collaborative studies; 4. To assess the psychological impact (including negative impact) of undergoing WGS testing on CP patients and families; 5. To refine the criteria to be used for assessing patient suitability for WGS testing in CP clinics; 6. To determine the effects of WGS testing on the clinical management of children with CP from the Paediatricians' perspective; 7. To generate recommendations for meeting the needs of patients and families undergoing WGS in the paediatric setting.

<table>
<tr><td>Study Endpoints</td><td colspan="2">1. Data on uptake of WGS testing in families with a child with CP
2. Data on the general diagnostic yield of WGS testing and the specific contributors to genetic disease in CP
3. Data on the clinical and psychosocial benefits, risks, and limitations of WGS testing for CP</td></tr>
<tr><td>Study Participants</td><td colspan="2">• non independent children under the age of 16 years with the clinical diagnosis of cerebral palsy (CP)
• Biological parent(s) of participating children</td></tr>
<tr><td>Sample Size</td><td colspan="2">66 children with CP and their biological parent(s) (up to 132)</td></tr>
<tr><td>Summary of eligibility criteria</td><td colspan="2">Inclusion criteria for children (proband or affected sibling):
1. Any sex, aged 0-16 years, inclusive
2. Has had a clinical diagnosis of cerebral palsy in medical record
3. Any GMFCS score (GMFCS 1-5)
4. Does not have a known genomic diagnosis that is thought to be causative of the CP phenotype
5. Has a legal guardian available to consent

Inclusion criteria for parent(s):
1. Biological parent
2. Aged 18 years or above
3. Is willing and able to give informed consent for participation in the study

Exclusion criteria
1. Children that have a pre-existing diagnosis from WGS or whole exome sequencing (WES)
2. Individuals not matching the inclusion criteria will be excluded</td></tr>
<tr><td>Planned Study Period</td><td colspan="2">Total length of the project: 2 years
Duration of a families' involvement: 15-16 months</td></tr>
<tr><td>Planned Recruitment period</td><td colspan="2">Start date: November 2022
End date: November 2024</td></tr>
<tr><td rowspan="5">Procedures:</td><td>Screening & invite</td><td>Clinical teams invite families to take part in the study at routine clinic appointments or by letter</td></tr>
<tr><td>Visit 1:</td><td>Informed consent
Family history
Arrangements made for blood sample collection from all participants
Completion of Pre-WGS testing parent/guardian questionnaire</td></tr>
<tr><td>Visit 2</td><td>Return of WGS results to families</td></tr>
<tr><td>Visit 3</td><td>Completion of Post-WGS testing parent/guardian questionnaire</td></tr>
<tr><td>Visit 4</td><td>Interview (optional)</td></tr>
</table>

3. FUNDING AND SUPPORT IN KIND

Funder(s)	FINANCIAL AND NON FINANCIAL SUPPORT GIVEN
Rosetrees Trust, Isaac Newton Trust, NIHR Cambridge BRC	Collectively these grants will support 3 genetic counsellors or research nurses (recruiting), 1 clinical scientist (DNA data interpretations), funding for 66 trio DNA sequences for the duration of the study.
Illumina	In kind support to facilitate project management and WGS interpretation using Emedgene and True Sight Software tools;
East Genomics Laboratory Hub	In kind support to facilitate WGS: • Extraction of DNA from blood samples • Dispatch of DNA samples and data to Illumina • Analysis and interpretation of WGS data (MDT) • Reporting WGS results to clinicians

4. ABBREVIATIONS

ACMG	American College of Medical Genetics
CCST	Cambridge Community Services NHS Trust
CI	Chief Investigator
CP	Cerebral palsy
CNV	Copy number variant
CRF	Case Report Form
DNA	Deoxyribonucleic acid
DOB	Date of Birth
DPA	Data Protection Act
EDTA	Ethylenediaminetetraacetic acid
EEA	European Economic Area
EGA	European Genome-Phenome Archive
EGLH	East Genomics Laboratory Hub
ESNEFT	East Suffolk and North Essex NHS Foundation Trust
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GLH	Genomic Laboratory Hub
GMFCS	Gross Motor Function Classification System
GP	General Practitioner
HPO	Human Phenotype Ontology

HRA	Health Research Authority
HTA	Human Tissue Authority
ICF	Informed Consent Form
ICMJE	International Committee of Medical Journal Editors
NGCP	Next Generation Children's Project
NHS	National Health Service
NIHR	National Institute for Health Research
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
SDHS	(University of Cambridge Clinical School) Secure Data Hosting Service
SNOWMED-CT	SNOWMED Clinical Terms
SOP	Standard Operating Procedure
TMF	Trial Master File
UK	United Kingdom
VUS	Variant(s) of Unknown Significance
WES	Whole exome sequencing
WGS	Whole Genome Sequence

5. BACKGROUND AND RATIONALE

Cerebral palsy (CP) is the most common cause of childhood-onset, lifelong physical disability in the world (1), and in the UK it is estimated that 1 in 400 babies born have a type of CP which, with a birth rate of approximately 700,000 per year, equates to as many as 1,700 new cases each year (2). It is estimated that lifetime care costs per CP patient is >£1m, and £1.7b per annum is spent on litigation for CP in the UK. CP can be defined as an umbrella term for 'a group of permanent disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing foetal or infant brain' (3). In addition to motor disorders, CP is often accompanied by disturbances of sensation, perception, cognition, communication, and behaviour, by epilepsy, and by secondary musculoskeletal problems (3). Many risk factors for CP are recognised, including prematurity, multiple births, placental pathology, intrauterine infection, and intrauterine growth restriction, neonatal stroke and hypoxic-ischemic encephalopathy. In most cases of CP, one or more of these factors is present, but for approximately a third of CP cases the aetiology remains unknown and evidence of perinatal sentinel events may be lacking (4). Clinical description, often based on observations of the child, is used to diagnose CP. It has been recommended that a diagnosis be confirmed by brain magnetic resonance imaging (MRI) in all cases, particularly when the aetiology is unknown (5). CP is a heterogenous condition in terms of the types and severity of impairments it encompasses which makes it important to further categorise individuals with CP into classes or groups. For the classification of CP, four major dimensions of classification are recommended: 1) motor disorder (the nature and typology of the disorder and a description of the functional motor abilities), 2) accompanying impairments, 3) anatomical and neuro-imaging findings, and 4) causation and timing (3). The Gross Motor Function Classification System (GMFCS) is a five-level classification that differentiates between children with CP based on a child's current gross motor abilities, limitations in gross motor function, and need for assistive technology, and it is widely used around the world (6).

With the advancement of genetic and genomic technologies, recent studies in other countries using whole exome sequencing (WES), whole genome sequencing (WGS) or copy number variant (CNV) analysis have identified genetic causes of CP in 10-31% of cases (7). So far there does not appear to be a significant association of genetic findings with any clinical risk factors, comorbid neurodevelopmental disorders, or motor phenotypes, suggesting that all CP cases should be offered genomic testing to avoid missed diagnoses (8–10). There is a growing list of genes and some CNVs with strong evidence for causing CP together with a large number of CP-associated genes that overlap with other movement and neurodevelopmental disorders (11). A recent WGS study on 150 children with CP identified variants in genes associated with hereditary spastic paraplegia, clotting and thrombophilic disorders, small vessel disease, and other neurodevelopmental disorders (12). The same study demonstrated the potential clinical impacts of a genomic diagnosis on a child with CP include eligibility for clinical trials, treatment modification with existing drugs, effecting a change in clinical management, as well as providing information about prognosis and risk for other gene-specific clinical features. There are also important potential impacts on family members, such as clarifying recurrence risk for parents in future pregnancies and estimating personal risk of other later onset disorders (12).

Following on from the 100,000 Genomes Project, the use of WGS in the National Health Service (NHS) for the diagnosis of rare diseases is gaining momentum. The Cambridge Next Generation Children's Project helped demonstrate efficacy of rapid turn-around time (TAT) WGS to diagnose intensively ill children leading to an NHSE rapid WES provision (R14) in NICU/PICU. Interestingly, this cohort included 15 subjects with CP of which 4 had a genetic diagnosis (27%).

The National Genomic Test Directory outlines the tests that are commissioned by the NHS in England with WGS currently available for 33 clinical indications, 55% of which are neurological disorders, but it does not currently include CP. There is scope for the Test Directory to be updated with new test targets or clinical indications upon the assessment of evidence showing clinical utility, unmet need, and benefits to patients (13). A UK effort now would harmonize with development/testing of a CP virtual gene panel (aka panel App) taking place in Australia, Canada, South Africa and the USA.

Requests for NHS WGS testing can be made to the Genomic Laboratory Hubs (GLHs) in England that are responsible for delivering the NHS Genomic Medicine Service. Currently, the NHS WGS is done by Genomics England/Illumina, who then feed the WGS data back to the GLHs for interpretation and reporting. Going forward, GLHs are developing in-house WGS pipelines, and we propose as a secondary technical objective to develop and enhance a complete rapid TAT WGS service for use in the NHS East Genomic Laboratory Hub (EGLH).

The NeuralNET is a programme of work with the ultimate goal of enhancing the early detection of brain and mental health conditions in time to intervene, which can potentially mitigate the progression of or even prevent disease onset. This will be achieved by a number of specific aims, the first of which is to optimise NHS diagnosis through rapid TAT WGS and stratified medicine. There has been investment in EGLH to provide infrastructure for large-scale, high-throughput sequencing capability and a collaboration between University of Cambridge, Illumina UK and EGLH have developed a scalable clinically-accredited bioinformatic pipeline for WGS. The EGLH now need to test the pipeline with prospective patients and this study has been designed to allow the EGLH to do this in a research safe haven. WGS for children with CP will be carried out in the first instance because CP is not currently a condition included in the Test Directory and testing these children will not conflict/compete with existing NHS clinical service provision. By testing children with CP, evidence can be gathered to potentially support an 'application' to have CP added as a clinical indication to the Test Directory whilst also providing information about the genetic contributors to CP in the UK population, and potentially enabling a genetic diagnosis for some of the children that could inform their clinical care.

The primary potential benefit to children who participate in the CP Pilot study is the identification of a genetic change that either caused or was a risk factor for their diagnosis of CP. For children who do receive a genetic diagnosis from the study, adjustments to their care or treatment may be available, and the family would also have information about the potential for other family members and future children to be affected with the genetic condition. The risks of participating in this study include the potential risk of emotional distress to children and their families as a result of discussing difficult topics, such as 'revisiting' a child's CP diagnosis. There is also a potential for emotional distress if a child receives a genetic diagnosis, as well as the potential for feelings of disappointment if a child does not receive a genetic diagnosis. We believe that the potential benefits to the participants justify the potential risks. We have also taken care to mitigate these risks as much as possible by providing detailed and accessible information about the study to families to allow them to make informed and voluntary decisions about whether to participate, by providing genetic counselling support to participants during the study, and by signposting to available local support and resources as needed during and after the study.

PPI/E: Whilst several studies into the prevalence of a genetic aetiology for CP cases using WGS and other genetic technologies have been carried out around the world, there are no published studies in a UK cohort. There is also very limited data on the acceptability of WGS testing in an UK CP population. A small survey with 18 children with CP and/or their families/carers was recently undertaken at the three sites to be involved in this study to demonstrate study feasibility and found that just over half (56%) of

respondents would take up an offer of genetic testing. This increased to 67% if the respondents who were undecided about WGS had their questions and concerns addressed (unpublished PPI data). This figure is in line with a recent study that looked at the attitudes and preferences towards genomic research of people with CP and their family's (14). It was also reflected by leadership of several CP patient advocacy groups (William Little Foundation, Cerebra).

6. OBJECTIVES AND OUTCOME MEASURES

This study has been designed to assess the feasibility of delivering a whole genome sequencing study in the NHS clinics to children with cerebral palsy. The study will test the feasibility of recruiting 66 patients from specialist CP clinics in the NHS and also the feasibility of using a <12-week turnaround time (TAT) WGS pipeline in the NHS to analyse and report WGS data. It will also generate information on the psychosocial impact of testing from the perspective of families/carers, and on the clinical utility of testing from a paediatricians' perspective. By meeting the study objectives (Table 1), the data generated from this study will be used to formulate a design for a larger study of 300 participants, including what eligibility criteria should be used and how to meet the needs of patients and families. As a consequence of assessing feasibility, it will also be established if genetic diagnoses can be made for children in the UK with CP in line with other genomic studies in other paediatric CP populations around the world.

Objectives	**Outcome Measures**
1. To test the feasibility of recruiting 66 patients from specialist CP clinics in the NHS for WGS and use a <12-week turnaround time WGS pipeline in the NHS to analyse and report the data	• Successful recruitment of 66 patients from specialist CP clinics in the NHS for WGS utilising a pipeline with a <12-week turnaround time for report of results.
1. To measure the uptake of WGS testing by families with a child with CP and to gain insight into families' decisions to decline WGS testing;	• Rate of uptake of WGS testing by families with a child with CP after consenting session with allied health professional • Clinician/site-reported aggregate information about why families declined to undergo genetic testing
2. To identify specific genetic contributors in child with CP recruited to this study;	• Number of children with CP receiving a genetic diagnosis • Details of genetic variants and the genetic diagnosis classification as per ACMG Guidelines
3. To identify potentially pathogenic variants of unknown significance in children with CP for further investigation of pathogenicity via follow-on collaborative studies;	Details of potentially pathogenic variants of unknown significance
4. To assess the psychological impact (including the negative impact) of undergoing WGS testing on CP patients and families;	• Decisional regret as measured on the validated Decisional Regret scale, assessing if there are any differences between families receiving a diagnostic result with those that do not receive a primary findings result • Parental/legal guardian expectations, experiences of and satisfaction with the consent and return of results appointments, perception of care received, the clinical, behavioural and psychosocial impact of the result and

	unexpected outcomes, as described by questionnaire and interview responses
5. To refine the criteria to be used for assessing patient suitability for WGS testing in CP clinics	Evidence supporting eligibility criteria required for NHS WGS testing for children with CP
6. To determine the effects of WGS testing on the clinical management of children with CP from the Paediatricians' perspective	Evidence regarding the clinical utility of WGS testing for children with CP
7. To generate recommendations for meeting the needs of patients and families undergoing WGS in the paediatric setting;	Evidence for generating recommendations for meeting the needs of patients and families undergoing WGS in the paediatric setting

Table 1 Study objectives and outcome measures

7. STUDY DESIGN

We aim to conduct a 2-year mixed-methods convergent parallel study design, using both quantitative and qualitative approaches, with 66 children who have a diagnosis of CP and their parents who are undergoing genomic testing using WGS. WGS uptake will be observed to establish the level of interest in this type of testing and the diagnostic yield from WGS testing in children with CP will be determined. In parallel, survey and interview outcomes will evaluate the families' experiences of WGS. The results will allow us to determine the feasibility of utilising an NHS, in-house WGS pipeline for delivery of a paediatric WGS service and to generate recommendations for eligibility criteria for testing children with CP and ensuring that the needs of the patients and families using this service are met. Participants will not be stratified for recruitment purposes; stratification will occur retrospectively prior to data analysis.

The study will be conducted at three sites: 1) the Peacock Centre (Community Paediatrics) at Cambridgeshire Community Services NHS Trust (CCST), 2) within Children's Services at East Suffolk and North Essex NHS Foundation Trust (ESNEFT), and 3) at the Great North Children's Hospital which is part of Newcastle Hospitals NHS Foundation Trust (Newcastle). Families will participate in the study for up to 16 months which will include three mandatory study visits and one optional study visit (see study flow chart – figure 1).

Visits can be in-person, or via phone/video call. Data will be collected via completion of questionnaires at visits 1 and 3, one questionnaire per family to be completed by a parent/legal guardian, and via interviews with a parent/legal guardian from 10 families per site (optional) at visit 4. All sample processing and analysis will be done under EGLH purview.

8. PARTICIPANT IDENTIFICATION

8.1. Study Participants

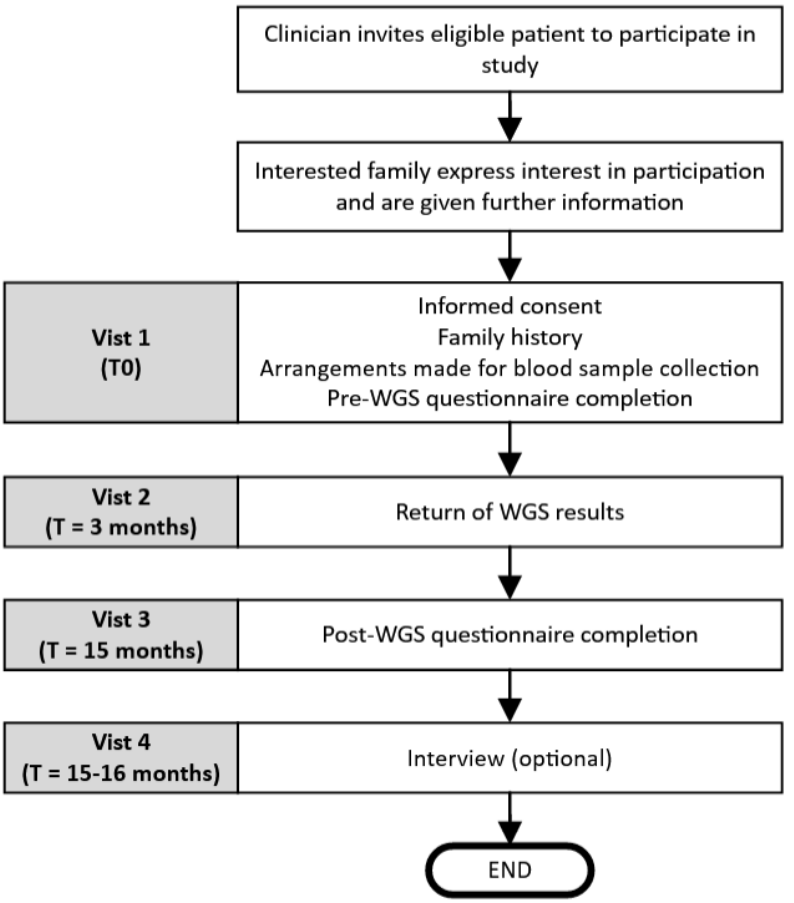

Figure 1 Study flow chart showing the sequence of study events and visits for a family

This study will recruit children (the 'proband') with CP and, wherever possible, both biological parents. Siblings affected with cerebral palsy can also be recruited. In this study, a 'family' is defined as the proband plus biological parent(s) and affected siblings if recruited. Children who have been adopted or where neither biological parent is available for testing can also be recruited.

8.2. Inclusion Criteria

Inclusion criteria for children (proband or affected sibling):

1. Any sex, aged 0-16 years, inclusive.
2. Has a clinical diagnosis of cerebral palsy in the medical record
    - GMFCS 1-5
3. Does not have a known genetic diagnosis that explains the CP phenotype
4. Has a parent/legal guardian available who can consent and is willing to complete study questionnaires

Inclusion criteria for biological parents having WGS:

1. Biological parent of proband
2. Aged 18 years or above
3. Is willing and able to give informed consent for participation in the study.

8.3. Exclusion Criteria

1. Children that have a pre-existing diagnosis from WGS or whole exome sequencing (WES) are excluded from the study.
2. Individuals not matching the inclusion criteria will be excluded.

9. PROTOCOL PROCEDURES

Procedures	Visit 1	Visit 2	Visit 3	Visit 4
	Day 0	+ 3 months	+ 15 months	+ 15-16 months
Informed consent	X			
Family history	X			
Blood tests	X			
Questionnaire completion*	X		X	
Results discussion		X		
Interview (optional)				X

Table 1 Schedule of study procedures. *Details of which measures will be undertaken at the visits is outlined in the summary of Questionnaire measures (table 2)

9.1. Eligibility Assessment

Clinicians at the three sites are responsible for assessing the eligibility of children prior to inviting families to participate. This study will report pathogenic or likely pathogenic findings that explain a child's CP which is characterised by a set of phenotypes unique to them. Since a child may also have a co-morbid condition (such as epilepsy or intellectual disability), that may not be explained the study genetic diagnosis, it is the clinician's responsibility to choose the most appropriate pathway for genetic testing for that child, be that via enrolment in this study, via NHS WGS-based testing for which the child is eligible based on their comorbid condition, or via both. The study team will be responsible for assessing the eligibility of parents once contact has been made with them. No exceptions will be made regarding eligibility; each participant must satisfy all the approved inclusion and exclusion criteria. Clinicians will be asked to keep a screening log of eligible children.

9.2. Recruitment

Participants will not be stratified by phenotype for recruitment purposes, but phenotype data will be utilised retrospectively for stratification prior to data analysis. Sites will invite eligible children to participate at least 1 month prior to a scheduled routine clinic appointment. Families will be contacted using the usual means of communication (letter/email/phone) for that family. Where a letter or email is sent, families/legal guardians will be given the study leaflet that briefly explains the study and the parent/guardian information sheet that provides a more detailed description of the study. Where parents/legal guardians are phoned, they will be told the same information that is on the letter/email and leaflet. Families/legal guardians will be asked to express interest in participating to the inviting clinician and interested parties will be asked to consent to be contacted by the research team which will be documented in the patient record. Where consent to contact is given, families/legal guardians will be asked to indicate their preferred method of communication and asked to provide contact details (telephone number and/or email address) which will be passed on to the study team. Families/legal guardians that were contacted by phone and who express interest in participating will subsequently be sent the study leaflet and parent/guardian information sheet and consent form. Sites will be asked to keep a record of the number of families invited and mode of invitation (letter/email/phone). They will

also be asked to record any reasons for not wishing to participate if given and report those reasons to the study team, in aggregate, when enrolment is complete. If a site was unable to contact a family/legal guardian prior to a routine clinic appointment, they can be invited at that appointment by providing a verbal description of the study and the study leaflet and parent/guardian information sheet and consent form.

The study team will contact the families using their preferred method of communication. A brief outline of the study will be given to families/legal guardians and any questions about the study will be answered. The involvement of the child plus parent(s) and affected siblings will be explained. If families would like additional time to consider their participation a follow-up call will be arranged or follow-up email sent. If the families/legal guardians wish to participate in the study, visit 1 date will be arranged. Wherever possible, visit 1 will occur immediately before or after a routine clinic appointment that the child attends in order to reduce inconvenience, but when this is not possible, for example when a parent who wishes to participate is unable to attend the appointment, the study team will liaise with a site to arrange a separate appointment or a telephone/video call appointment. Telephone/video call appointments for visit 1 can be conducted for the informed consent procedure and pre-WGS questionnaire completion however participants will need to attend an appointment for the blood sampling procedure, either with the study team, at outpatient phlebotomy service, or GP surgery.

Where biological parents do not live together we will attempt to contact them both, with the appropriate permissions from each.

For families that do not wish to participate, they will be thanked for considering the study and asked if they would be willing to provide a reason as to why they don't want to participate if not already given.

9.3. Informed Consent

Informed consent will be discussed by a member of the research study team at visit 1 which will be held face-to-face or via telephone/video-call.

The consent discussion will include a description of:

- Study procedures in detail
- What to expect, e.g., time frame for reporting to clinicians, possible diagnosis, lack of diagnosis
- Limits of the study such as non-reporting of incidental findings irrelevant to acute presentation and care
- The study being of minimal medical risk (one blood draw only)
- Risks including the potential for study results undermining a legal case of medical negligence that is in progress or a family might want to pursue, and the potential to reveal non-paternity.

Written Informed Consent will be obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent. The person who obtained the consent will be suitably qualified and experienced and have been authorised to do so by the Chief Investigator. All research team members will have undertaken Good Clinical Practice (GCP), Good Research Practice and consent training. A copy of the signed Informed Consent will be given to the participant and a copy will be filed in the participant's medical record. The original signed form will be retained at the study site.

Prior to visit 1, families/guardians will be sent consent documentation for all potential participants including information sheets for the child appropriate to their age, for parents/legal guardians

consenting for children, and for adult participants (if biological parent(s) are available and wish to participate). Copies of consent forms and assent forms will also be sent and families will be encouraged to take the time to read all the study information before attending the visit. The consent documentation presented to the participants will detail no less than: the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that participants are free to withdraw from the study at any time for any reason without prejudice to future care, without affecting their legal rights, and with no obligation to give the reason for withdrawal.

Families will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their clinician, or discuss with other independent parties to decide whether they will participate in the study. Where families wish to participate in the study, a parent/guardian must personally sign and date the latest approved version of the Parent Consent Form on behalf of the participating child(ren) before any study specific procedures are performed. Participating biological parents must also personally sign and date the latest approved version of the Informed Consent Form for themselves. Additional Assent will be sought from children wherever possible.

Where visit 1 is carried out via video or telephone call, families will be asked to complete an electronic versions of the consent forms.

At subsequent visits with families, a verbal consent check will be done prior to any study procedures being carried out.

During the course of the study, if a proband reaches the age of 16 and they have capacity, they will be asked to reconsent to the study. Where a proband is deemed to lack capacity, their nominated legal representative will be asked to reconsent to continue in the study.

9.4. Description of study procedure(s) for consented participants

Description of questionnaire measures

The questionnaires will collect demographic information and will also include questions that are standardised, peer reviewed, published and validated for use in research, as well as some study-specific questions (Table 2). The questionnaires will be online and will permit save and return to the data entry, permitting completion over several visits.

Survey Domain	Description	Visit 1	Visit 3
Participant characteristics and personality traits			
Demographics	Child age, parent/guardian age, gender, parent/guardian education, parent/guardian employment status, number of children in household, ethnicity, postal code of child's primary residence	x	
Intolerance for uncertainty	Short version of the Intolerance for Uncertainty scale. A 12-item measure for assessing intolerance for uncertainty. Items are rated on a 5-point Likert scale (15)	x	
Attributes of informed decision-making			

Attitude	Four-item scale examining general attitudes to genome sequencing e.g. harmful – beneficial, unimportant – important, measured on a five-point Likert scale (16)	x	x
Self-reported informed decision-making	Question used previously in survey on genome sequencing in the 100,000 Genomes Project "Did you have enough information and discussion with doctors or other healthcare providers to make an informed choice about having whole-genome sequencing?" (16)	x	
Decisional conflict	Sixteen-item measure with five-point Likert scale which assess decisional certainty or conflict about a healthcare decision (17)	x	
Clinical, psychosocial, behavioural outcomes			
Decisional regret	Five-item measure with five-point Likert scale which assesses regret or remorse about a healthcare decision, with scores ranging from 0 to 100. DRS scores can be defined into three categories: no decision regret (DRS score 0), mild decision regret (DRS score 1–25), and moderate to high decision regret (DRS score >25) (16)		x
Parental empowerment	Genomics Outcome Scale: six-item questionnaire with five-point Likert scale which captures the theoretical construct of empowerment relating to genomic medicine (18)	x	x
Psychological impact	Adapted 12-item version of the Feelings About genomic Testing Results (FACToR) with five-point Likert scale which measures the specific impact of result disclosure after genomic testing (19)		x
Family impact	PEDS-QL Family impact module: sixteen-item questionnaire with five-point Likert Scale which explores problems with communication, worry, daily activities, family relationship (20)	x	x
Clinical, social and behavioural impact of results	Study specific questions which explore: changes to clinical management, understanding the likely course of the condition, changes to child's/family's lifestyle, connecting with specific rare disease support groups/other families, communication with medical professionals, reproductive decision-making and identification of other at-risk family members. Each item will have 5 levels (not at all – a great deal).		x
Diagnosis-dependent services and support			
CP diagnosis-dependent community	*Does child/family receive financial support, social services, community services, etc. based on the child's diagnosis?*	*x*	

services and/or financial support			
CP diagnosis-dependent social support	*Is child/family a member of a CP-specific support group/charity/organisation?*	*x*	

Table 2 Summary of survey measures used in the questionnaires (adapted from (21))

Visit 1

Where possible, a family history will be collected from the parent/guardian, and will include drawing a family tree (pedigree diagram) on paper. The pedigree diagram will only contain identifiable information relating to study participants. All other information will be anonymised.

The pre-WGS questionnaire will be administered by the study team electronically on a study laptop during appointments held face-to-face. Only one pre-WGS questionnaire needs to be completed per family. For family appointments held virtually, depending on family preference, they will either be sent a link during the appointment to complete the online pre-WGS questionnaire electronically, or they will have been sent a paper version of the pre-WGS questionnaire and asked to complete it, sending it back to the study team with the signed consent forms. The survey measures administered at visit 1 are described in Table 2. Visit 1 will last approximately 60 minutes.

Visit 2: Return of results

As part of the study, parents/legal guardians of children with CP consent to receive the results of the WGS testing. The following results will be reported:

1. Positive - Variant(s) identified which are thought to be partially or fully causative of the CP phenotype, and which are classified as pathogenic (Class 5) or likely pathogenic (Class 4) following application of ACMG variant classification guidelines
2. Negative – No clearly pathogenic or likely pathogenic variants identified which are partially or fully causative of the CP phenotype

To facilitate a rapid WGS TAT, incidental and secondary findings that are not associated with the primary reason for referral will not be reported which is in line with other genomics research projects currently ongoing in the UK.

The referring clinician or a member of the referring clinician's team is responsible for reporting results to families. Once results have been reported to families, the clinician/clinical team must inform the study team within 7 days so that they can calculate when visit 3 is due.

Children receiving pathogenic/likely pathogenic findings will be referred onward by the referring clinician to local clinical genetics teams and/or other clinical specialists for management as appropriate. Families will be followed-up by the study team at 6 months post result to the check on status of any onward referrals make by the clinical team, and to recommend that they contact their clinician if they haven't heard/received anything or if they have any questions about their child's clinical management.

Visit 3

One week in advance of the visit 3 time point which occurs 12 months after visit 2, families will be contacted using their preferred method of communication and asked to confirm their consent to

continue in the study. For families who are happy to continue, depending on their preference, they will either be sent a link to an online version of the visit 3 post-WGS questionnaire and asked to complete this electronic version, or they will be sent a paper version of the post-WGS questionnaire and asked to complete it, sending it back to the study team in a pre-paid envelope. We will ask that the same person who completed the pre-test questionnaire completes the post-test questionnaire to maintain internal consistency. The survey measures administered at visit 3 are described in Table 2. The link to the online survey will be sent on the date of visit 3 and paper versions will be posted two working days before the visit 3 date. Families will be asked to complete the post-WGS questionnaire as soon as possible. If completed surveys/questionnaires have not been received within 2 weeks of having been sent, a follow-up reminder email will be sent, or a phone call made. For families who need assistance in completing the post-WGS questionnaire, options will be available which include telephone advice on how to fill out the forms, or a telephone/video call interview to complete the forms if this is preferred. Time required to complete the post-WGS questionnaire is approximately 20-30 minutes.

Visit 4

When families are contacted one week prior to the visit 3 date, they will also be asked if they are interested in participating in an interview. Taking part in the interview is optional. Families will be given an outline of what the interview will involve and the types of questions that will be asked. If a family is willing to be interviewed, a 30-45 minute phone or video-call (Zoom) appointment will be arranged, depending on family preference, on a date within 13 months of visit 2. Interviews will be carried out in a single session but can be offered as two shorter sessions if there are unexpected interruptions (due to parenting/caring responsibilities) or in circumstances where this is preferred by the parent/guardian. All family members participating in the study can take part in the interview should they wish to. Child participation is at the discretion of the parent(s)/legal guardian(s).

The post-test qualitative interview will be semi-structured, using a topic guide (Appendix 1) to direct discussion. The interviews will be recorded (audio-only) to allow for subsequent transcription by the study team. The topic guide has been informed by the literature. Specific prompts will be informed by the advisory team comprised of a genetic counsellor, a behavioural scientist, community paediatricians, and a patient advocate. The topic guide and prompts have been developed by the study team, and will be refined after the first few interviews have taken place. The topic guide has been designed to explore family experience of CP including journey to diagnosis, attitudes and beliefs about genetics of CP, experience of recruitment and consent process for WGS, expectations of WGS for CP, experience of communication of results, perceived clinical impact of WGS on child and family, perceived psychological impact of WGS on child and family, impact on any CP diagnosis-based support, family's advice for other families of children with CP considering WGS, and family's advice for health professionals offering WGS for children with CP.

9.5. Unique study ID allocation

Study participants will each be allocated a unique study ID that follows the format NNETxyyyzz, where x will be the numerical site code (1 = CCS NHS Trust, 2 = ESNEFT, 3 = Newcastle), yyy will be the proband number allocated sequentially per site, and zz will be sequential numbers (01, 02, 03 etc.) allocated to participants belonging to the probands' family. For z, 01 will always be the proband (child with CP), where parents are also recruited, 02 will always be the mother, 03 will always be the father, and 04 will always be a sibling.

For example,

i. NNET200101 will be the participant number for a proband/child with CP recruited by ESNEFT.
ii. NNET300503 will be the participant number for the father of participant NNET300501, both recruited by Newcastle

9.6. Sample Handling

9.6.1. Sample Collection

In order to undergo WGS testing, each study participant is required to provide a suitable DNA sample (from venepuncture). For each participant, DNA will either be extracted from a blood sample specifically collected for the study or, for child participants only, if a suitable stored DNA sample is available in an accredited NHS Genomic Laboratory Hub, a request will be made to use an aliquot of it for WGS. Blood samples will be collected by a suitably qualified and experienced person following local guidelines after informed consent has been given at visit 1. Local site procedures will be followed regarding the arrangement of paediatric blood tests, including the provision and application of anaesthetic cream prior to visit 1 if required.

Since blood samples will be sent to the East Genomics Laboratory Hub (EGLH), an accredited NHS laboratory service, for processing and storage, their requirements for blood sample collection and transportation of samples for rare and inherited disease WGS testing will be followed.

Blood sample requirements

Child: 1-5ml blood in an EDTA blood tube, volume dependent on age of child and difficulty of draw; a minimum of 1 ml will be needed.
Adult: 5ml blood in an EDTA blood tube

As participant blood samples will be processed by EGLH, they must be labelled with the participants' name (first name and surname), date of birth and NHS number / hospital number. A specimen barcode label that encodes the external specimen ID can be used and is recommended to ensure it can be scanned without the opportunity for human transcription error.

9.6.2. Sample transportation and processing

Each participant blood sample must be transported to the Cambridge University Hospitals Genomic Laboratory, part of EGLH, accompanied by a referral form, completed by the study team.

Samples stored at room temperature should be delivered by a member of the study team to the EGLH Genomics Laboratory Specimen Reception or be sent using either Royal Mail or a designated approved courier (e.g., ESNEFT routinely sends samples to EGLH). If samples are sent by Royal Mail they must comply with Packing Instructions 650 for category B substances. **Further details are available on the EGLH website (https://www.eastgenomics.nhs.uk/for-healthcare-professionals/genomic-tests/transport-of-samples/).**

Blood samples will be processed to extract the DNA which will then be stored according to EGLH SOPs. The samples will be stored long-term by EGLH as per NHS DNA samples and will be available for future NHS genetic testing purposes. DNA will also be available for other future ethically approved studies.

9.6.3. WGS, data analysis, interpretation and reporting

The study team will be responsible for ensuring that a DNA sample for each participating family member is available before triggering the WGS process. De-identified participating child and family member DNA samples will be securely transferred to Illumina in barcoded tubes along with de-identified phenotype information corresponding to each sample that is necessary to analyse the sequence data. WGS (over 30× coverage in aligned reads), quality control, read alignment to the human reference genome build 38, and variant calling using the DRAGEN pipeline will be performed by Illumina. WGS Data will be returned to the EGLH for analysis using the Illumina TruSight Software Suite (TSS). Genetic variants will be analysed and interpreted to identify the molecular cause of the child's CP following standard EGLH processes and policies. Only pathogenic or likely pathogenic findings that explain the child's phenotypes which characterise their CP will be reported. Variants will be interpreted and reported following the latest international variant interpretation guidelines (American College of Medical Genetics and Genomics/Association for Molecular Pathology) and national guidelines (Association of Clinical Genomic Science). If both parents are available, gene agnostic analysis will be carried out, but a CP-specific internationally agreed virtual gene panel will be applied to filter genetic variants for analysis in singletons and duo's. Candidate pathogenic and variants of uncertain significance will be discussed in multidisciplinary team meetings (MDTs). Variants of uncertain significance may also be discussed at MDT to inform on any further testing that may provide additional evidence for pathogenicity or classify the variant as benign. Prior to reporting, pathogenic and likely pathogenic variants will be confirmed using orthogonal methods within the EGLH laboratory. A research report will be issued to the referring clinician for the WGS analysis, alongside a clinical report confirming any pathogenic or likely variants. Variants of uncertain significance will not be reported. Genetic findings that are not associated with the primary reason for referral ('incidental/unexpected findings') will not be reported. Pathogenic, likely pathogenic and selected variants of uncertain significance will be submitted to the public variant database ClinVar, with pathogenicity and high level phenotype information. Variants in candidate genes of uncertain significance may be submitted to GeneMatcher.

9.7. Collection of participant personal details

In order to:

1. Provide feedback of pertinent findings;
2. Capture information from participating families by self-reporting (e.g. through questionnaires);
3. Retrieve information from electronic and paper-based health records, and
4. To be able to contact families inviting them to join follow-up studies related to the WGS findings (which will have their own, separate REC-approved patient information sheets and consent forms)

families will be asked to provide the following details for each participant:

1. Surname
2. Given names
4. Date of Birth (DOB)
5. Sex at birth
6. Home Address
7. NHS number
8. Phone number
9. Email address

All data will be kept in the Study Link Table, where it will be linked to participants' unique study ID numbers. Access to the Study Link Table will be restricted to the CI and study coordinators for the purpose of the above activities.

9.8. Collection of clinical/phenotype data

In 2022, the International Cerebral Palsy Genomics Consortium (ICPGC) defined 107 common data elements (CDEs) with the purpose of standardising phenotype data capture in CP genomic studies (22). Clinical/phenotype data capture for this study will utilise some of these CDEs and include other data elements pertinent to achieving the aims of this study. Following visit 1, the referring clinician will be asked to provide clinical/phenotype data for the proband. Clinical/phenotype data will be entered by the clinician into an online questionnaire delivered by Qualtrics. The study team will use the secure NHS.net email system to contact clinicians requesting data entry, sending them details (name, date of birth, NHS number, study ID number) of who to complete the online questionnaire for. The child's study ID number will be entered into the questionnaire and also their first name and year of birth as a check to ensure that information has been entered for the correct child. No other personal details will be entered. Where possible, the clinical/phenotype data will be enriched by coding the clinical symptoms and signs with terms from the Human Phenome Ontology (HPO) database, SNOMED Clinical Terms (SNOWMED CT), and ICD10 terms.

9.9. Collection of clinical utility data from clinicians

The referring clinician will be asked to provide clinical utility data for the proband and their family at 12 months post results disclosure date (i.e. post Visit 2 date). The referring clinician will be sent a link to an online questionnaire delivered by Qualtrics. The study team will use the secure NHS.net email system to contact clinicians requesting data entry, sending them details (name, date of birth, NHS number, study ID number) of the child to complete the online questionnaire for. The child's study ID number will be entered into the questionnaire and also their first name and year of birth as a check to ensure that information has been entered for the correct child/family. No other personal details will be entered. We will be using C-GUIDE™ (licensing agreement pending), which is a validated outcome measure to assess the clinical utility of genetic testing from providers' perspectives (23). C-GUIDE™ is designed to capture the clinical utility of a genetic test as it relates to (i) understanding diagnosis and prognosis, (ii) informing medical management, (iii) awareness and actionability of reproductive and health risks for patients and family members, and (iv) patient and family well-being.

9.10.Withdrawal of Participants

During the course of the study participants may choose to withdraw from the study at any time. This may happen for several reasons, including but not limited to their inability to comply with study procedures, and participant decision.

Participants may decide to withdraw from the study completely, and withdraw the data and sample collected up until the point of withdrawal. The data and samples already collected would not be used in the final study analysis. Alternatively, participants may decide to withdraw from the study but permit the sample and/or data obtained up until the point of withdrawal to be retained for use in the study analysis. No further data or samples would be collected after this partial withdrawal. If participants wish to withdraw, the options of withdrawing the DNA sample and the various types of data will be discussed.

There will be an SOP outlining this process and various options for withdrawal of data and /or DNA samples. The type of withdrawal and reason, if given, will be documented.

9.11. Definition of End of Study

The end of study is the date of the last visit of the last participant/family. This could be visit 3 or visit 4 depending on whether the family have agreed to be interviewed.

10. CO-RECRUITMENT TO NIHR BIORESOURCE RARE DISEASES STUDY

At sites where the NIHR BioResource Rare Diseases (REC reference 13/EE/0325) has been approved, families (child and biological parent(s)) will be invited to participate in that study as well. Recruitment to both studies can occur at visit 1 or at another time if that is preferable to the family; there are no subsequent visits for the NIHR BioResource Rare Diseases study. Families will be sent the relevant participant information leaflet(s) and consent form(s) for the study to read in advance of visit 1 and given details of who to contact should they have any questions or want further information. Families have the option of either just the child with CP or both the child with CP and participating biological parent(s) taking part. If families wish to participate in the NIHR BioResource Rare Diseases study, members of the NeuralNET study team trained and approved to recruit to the study will recruit families following the recruitment procedures outlined in the study protocol.

11. DATA MANAGEMENT

Institutional guidance on research data management will be used to ensure adherence to guidelines and best practice.

The CI, or delegated members of the study team, will maintain an electronic trial master file with access restricted to authorised members of the study team. Sites will maintain an electronic study site file with access restricted to individuals who are on the delegation log for that site.

On all study documents, other than the signed consent form, the participant will be referred to by a unique study ID number, not by name. The pseudonymisation of data will be enabled by the use of the Study Link Table, a spreadsheet linking participant identifiable information to their unique study ID number. The Study Link Table will be stored in the Safe Haven at the University of Cambridge Clinical School Secure Data Hosting Service (SDHS).

Any paper versions of consent Forms, questionnaires and pedigree diagrams will initially be stored in a secure, locked location and will be accessible only to the members of the study needing to use them in fulfilling their role. Before the end of the study the consent forms will be scanned and saved in electronic form in the SDHS. The paper documents will then be destroyed as confidential paper waste. The purpose of the pedigree diagram is to aid WGS data analysis and interpretation. The family history information from the paper version of the pedigree diagram will be entered electronically into PhenoTips™, online pedigree software used by EGLH and accessed via the NHS IT network. The PhenoTips™ record will be stored securely on the EGLH network where it will be accessible to the clinical scientists carrying out the WGS analysis and interpretation. Once the family history information has been entered into PhenoTips™, the paper version of the pedigree diagram will be destroyed as confidential waste.

The pre- and post-WGS questionnaires in will be administered online via the Qualtrics web platform. Data will either be entered by families directly into Qualtrics or responses given by families on paper

forms will be entered into Qualtrics by a member of the study team. Where the data is entered into Qualtrics by a member of the study team, another member of the study team with permission to view the information will check it to ensure that there are no data entry errors. The paper forms will be destroyed as confidential paper waste once the check has been undertaken. Research data collected in Qualtrics will be automatically deposited in the SDHS and at the end of the study, the pseudo-anonymised data will be pushed to the standard University of Cambridge network for analysis.

Pseudo-anonymised clinical/phenotype data from clinicians will be collected in Qualtrics from where it will be automatically saved in the Safe Haven at the SDHS. HPO data relevant to the analysis and interpretation of the WGS data and reporting of WGS results will be securely transferred to EGLH, where it will be stored in PhenoTips along with the pedigree diagrams.

EGLH will pseudo-anonymise data (DNA sample data and clinical data, including HPO terms) being shared with Illumina. Upon completion of the study, Illumina will destroy any left-over samples and delete any copies of the WGS data that were retained by them until the end of the study for evaluation purposes. All EGLH data, including DNA sample data and HPO terms, will be stored indefinitely as NHS data on cloud-based systems according to standard NHS and EGLH processes and policy. EGLH will provide the study team with a pseudo-anonymised list of variants (pathogenic, likely pathogenic and/or VUS) for each proband that will be stored on the standard University of Cambridge IT network. The data will not be associated with any other study data. The variant information is needed for publication purposes and to identify variants that require further investigation via future ethically-approved studies.

The WGS data will be stored at the University of Cambridge Secure Computing Research Platform (SRCP), in Very High Impact (Level 3) security storage which is for sensitive or confidential data. The SRCP is an ISO27001 Certified Platform Service adhering to the highest standards of data privacy and security and is compliant with the NHS Data Security and Protection Toolkit. It provides a secure, isolated, and controlled environment for data storage, processing, and analysis that has access controls (strong authentication, authorization controls, and access logging) to manage access to the platform. Access to the study data in the SRCP will be controlled by the CI in conjunction with the study bioinformatician. The WGS data will be pseudoanonymised and the study link table that identifies participants will be kept in an entirely separate secure system (the University of Cambridge Clinical School SDHS). Individual members of the study team will not have access to both systems to maintain confidentiality of participants. WGS data will be stored in the SRCP until the end of the study and not longer than 5 years.

The interview audio recordings collected at visit 4 will be stored securely in the SDHS. The transcription will be carried out internally by the study coordinators/genetic counsellors, and the pseudo-anonymised transcripts will be saved on the standard University of Cambridge network for analysis purposes. To ensure transcription accuracy and consistency, 20% of the recordings will be double coded. Following completion of data analysis, the interview audio recordings will be deleted.

At the end of the study, all data sets except for the WGS data will be collated into a master dataset through the use of the unique study ID numbers for analysis purposes.

11.1. Data sharing

Oversight of data sharing will be by the study coordinators and will be aligned with permissions granted in the informed consent. Where a parent/legal guardian has consented, anonymised variant and phenotype data will be uploaded to the DECIPHER database. EGLH routinely deposit pathogenic variants

identified from all forms of genetic testing into ClinVar. EGLH will deposit anonymised pathogenic variants identified in this study into ClinVar, along with the interpretation rationale and some high level phenotype data e.g. a clinical diagnosis of CP.

Where participants are co-recruited to the NIHR BioResource Rare Diseases study, the study bioinformatician will transfer WGS and phenotype (HPO terms) data to the NIHR BioResource using a secure method. The NIHR BioResource will store the data securely on the Cambridge HPC and their Data Access Committee will control further access to and use of the data as outlined in their protocol. Where participant WGS data is transferred to the NIHR BioResource, the WGS data will be deleted from the SRCP.

Anonymised data will be made accessible to bona fide and authorised medical researchers upon request at the discretion of the chief investigator. We may send anonymised data collected as part of the study (e.g. questionnaire responses, genomic findings, interview transcripts, DNA) outside the European Economic Area (EEA). The data can only be used by organisations and researchers to conduct research in accordance with the UK Policy Framework for Health and Social Care Research, and the participants' data could be used for research in any aspect of health or care.

11.2. Data archiving

At the end of the study, all electronic study data (including the TMF, master data sets and Study Link Table) will be archived for 10 years. Where data contains identifiable information, it will be stored in the SDHS. All other data will be stored in a Department of Paediatrics area on the standard University of Cambridge network. Prior to archiving, all files will be locked (made read-only) and password-protected if this has not already been done. The CI will be responsible for the archived data. Sites will be asked to archive their electronic site files for 5 years. WGS data will be stored securely in the SRCP until the end of the study and not for longer than 5 years.

12. STATISTICS AND ANALYSIS

This is a pilot study with total of 66 trios. Each site will be asked to recruit 22 children/trios. Each site has a caseload of approximately 180 paediatric CP patients which is a sufficient population size to achieve the recruitment target since only 1 in 8 of their caseloads can be involved. Previous studies (7) have found that 10-31% children with CP have a genetic cause and, using these percentages, it is expected that 7-20 children in this study will have a genetic finding associated with CP.

This convergent parallel mixed-methods study will collect quantitative and qualitative data which will initially be analysed separately but integrated at the point of interpretation.

We sought informal review of the protocol by two other researchers experienced with similar study designs, and they have recommended a descriptive approach to the quantitative data. Given the small sample size in this pilot study, they felt that it is unlikely that there will be sufficient numbers to allow inferences about correlations of demographics or phenotypic data with outcomes. Therefore, frequencies, means and standard deviations of quantitative date will be calculated and descriptive statistics will be reported with 95% confidence intervals. Participants will be stratified for post-hoc analysis by factors such as the presence or absence or risk factors, co-morbidities, and by clinical phenotype.

Qualitive questionnaire and interview data will be analysed using QSR NVivo 12. All transcripts will be reviewed and coded by two researchers to allow for full data immersion. Our analysis will be based on critical realism paradigm, which assumes that individuals experience the same objective 'reality' but do so in different ways (24). We will utilize an inductive approach to thematic analysis as a methodological framework. This will allow theories to be applied flexibly without a priori theoretical assumptions about what might be learned from the data (25).

13. PROTOCOL DEVIATIONS

A study related deviation is a departure from the ethically approved study protocol or other study document or process (e.g. consent process or administration of study intervention) or from GCP or any applicable regulatory requirements. Any deviations from the protocol will be documented in a protocol deviation form and filed in the study master file.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1. Summary of Potential Benefits and Risks

The primary potential benefit to children who participate in this study is that it might identify a genetic change that either caused or was a risk factor for their diagnosis of CP. For children who do receive a genetic diagnosis from the study, there are subsequent potential benefits. One potential benefit of receiving a genetic diagnosis would be to obtain information about the child's prognosis and whether there is a possibility of other problems developing which are associated with the diagnosis, informing appropriate clinical care and support. A child receiving a genetic diagnosis may also become eligible to take part in clinical trials, or to have their treatment personalised with the addition of existing therapies for that genetic condition. The child and family may also be able to take advantage of support groups or organisations specific to the child's genetic diagnosis. Another potential benefit to both the child and the family from receiving a genetic diagnosis is to obtain information about the likelihood that other family members or future children could either be affected with or pass down the genetic condition, which could inform medical care and/or future reproductive decision-making.

There are potential risks to the child and biological parent(s) as part of the study associated with blood sample collection which could include pain/discomfort during the test, the chance of subsequent bruising, or the child may become distressed. There is a risk of emotional distress to participants and families from discussing and/or answering questions about difficult topics such as the family's experience surrounding the CP diagnosis, illness in family members, and/or difficult family relationships. There is also a risk of emotional distress from waiting for and/or receiving genetic test result, as well as a risk of emotional distress from receiving and adjusting to a genetic diagnosis. There is a potential risk that a study result could undermine a legal case of medical negligence that a family has in progress or might want to pursue. There is also a risk of WGS revealing to participants genetic relationships not previously known and/or disclosed (e.g., non-paternity, adoption).

Measures will be taken to minimise the potential risks as much as possible, and clear information about the potential risks will be included in participant information materials and will be reviewed with participants by the study team. Only appropriately trained staff will perform phlebotomy, and for children numbing cream/spray will be used and play used as a distraction technique. Throughout the informed consent discussion and during any conversations regarding the study, the study members will check in with participating family members to make sure they are comfortable to continue. Participants

will also have the option to decline to answer any question, or to pause or stop the conversation with the option to continue at a later time. If the participant experiences significant emotional distress during a study visit, or if a study member becomes aware that the participant is suffering from persistent emotional distress after the visit, the study member will signpost the participant to their GP to seek advice about their mental health concerns and onward referral to local support resources as appropriate.

A member of the study team will be available as needed for support during the result disclosure visit and for the remainder of the study. For children who receive a genetic diagnosis, the clinician and/or study team members will provide the participant and family with any support resources specific to the genetic diagnosis of which they are aware. It will be recommended by the study team that any participant who receives a new genetic diagnosis from the study be referred by their clinician to their local Genetics Service for further advice and support.

14.2. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

14.3. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

14.4. Approvals

Following Sponsor approval, the protocol, informed consent form, participant information sheet, poster and leaflet will be submitted to an appropriate REC, Health Research Authority (HRA) and host institutions for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

14.5. Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, HRA, host organisation, Sponsor and funder (where required). In addition, an End of Study notification and final report will be submitted to the same parties.

14.6. Participant Confidentiality and Data Protection

The study will be guided by the appropriate sections of the NHS IG Toolkit, ISO27001, data protection legislation (General Data Protection Regulation (GDPR) and Data Protection Act 2018 (DPA 2018)), the Information Commissioner Office recommendations and best practice in IT security technologies. GDPR/DPA 2018 requires data to be de-identified as soon as it is practical to do so. The processing of the personal data of participants will be minimised by making use of a unique participant study number only on all study documents and any electronic database(s) immediately following consent. All documents, both paper and electronic, will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants' personal data.

DNA samples will be stored in a secure manner by EGLH and in accordance with the DPA 2018, GDPR and the Human Tissue Authority (HTA).

Qualtrics is a web-based application used for building and managing online surveys and databases widely used by researchers in the University of Cambridge. Access to Qualtrics via the University involves 2-factor authentication. Using just their participant study ID number and initials, participants will complete the study questionnaires online, or members of the study team will enter data from paper questionnaires in to the online questionnaires, and the data will be fed directly into the safe haven in the SDHS.

The Study Link Table, electronic copies of consent forms, interview audio files, and family questionnaire data and phenotype data, both from Qualtrics, will be stored on the SDHS. The SDHS provides a dedicated network, separated from the production network by a firewall, for storing sensitive personal data and hosting computers involved in its management and analysis. The SDHS is registered under the School of Clinical Medicine's NHS Digital Data Security and Protection Toolkit. All equipment connected to the SDHS must be located in the University of Cambridge's Clinical School Computing Service's physically secure server rooms. Access to the SDHS is authorised by the University of Cambridge School of Clinical Medicine Information Governance Officer and is provided by secure Virtual Desktop (based on Citrix XenDesktop 7.6).

14.6.1. Contacting participants using Zoom

Where families would like to discuss the study or do the study visits via video call, Zoom will be used.

The study team hold their own 'Pro' Zoom account. To ensure the security of the online calls/visits, when the calls are set up the study team will:

- Password protect the meeting
- Meeting ID will be randomly generated
- Disable 'join before host' and enable 'waiting room'
- Once the participant is on the call the meeting will be 'locked' to prevent unauthorised access
- Disable 'screen sharing'

To ensure that confidentiality is maintained, participants will be advised that the platforms calls are encrypted, but it is their responsibility to ensure they have adequate anti-virus protection on their computers. They will be made aware that some personal information from their Zoom account is stored locally on the computer (this is particularly important if participants intend to use public or shared computers).

Participants will have the option of doing Visit 4 by phone or on Zoom. Where visit 4 is on Zoom, it will be recorded and saved to the SDHS.

14.7. Expenses and Benefits

Upon completion of the study, either after visit 3 or visit 4 if a parent/guardian agrees to be interviewed, a payment of £30 will be made per participating child.

15. FINANCE AND INSURANCE

15.1. Funding

This study is funded by the Rosetrees Trust, Isaac Newton Trust, and NIHR Cambridge BRC and will receive support in kind from Illumina (WGS) and EGLH (DNA extraction, storage and WGS data analysis, interpretation and reporting).

15.2. Insurance

The University of Cambridge will provide an indemnity to pay non-fault damages or compensation to participants who suffer bodily injury caused by their participation in this study.

15.3. Contractual arrangements

Appropriate contractual arrangements will be put in place with all third parties.

16. PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, conference presentations and any other publications arising from the study. Authors will acknowledge that the study was funded by the Rosetrees Trust, Isaac Newton Trust and NIHR Cambridge BRC and supported by Illumina and EGLH. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

Families will be asked at visit 1 if they would like to receive a summary of the study findings and/or a link to the full report/publication at the end of the study.

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18. APPENDICES

18.1. APPENDIX 1: Post-test Qualitative Family Interview Topic Guide

Family Interview Topic Guide

Family experience of cerebral palsy (CP)

- How old was your child when they were diagnosed with CP?
- How long did it take for them to receive confirmed diagnosis?
- How did the diagnosis process affect you and your family?
- How do you feel that your child's diagnosis of CP has affected your child's life? How has it affected your life and your family's life?

Beliefs about the cause of their child's diagnosis of CP

- Prior to finding out about this study, what were your thoughts about potential cause(s) of your child's diagnosis of CP?
    - Had you previously considered that your child's diagnosis of CP may have a genetic cause?

Experience of recruitment and consent process for whole-genome sequencing (WGS)

- How did you first find out that your child could have whole-genome sequencing (WGS) for CP as part of a research study?
- How did finding this out make you feel? How do you think it made your child feel? How do you think it made other members of your family feel?
- What went well with the recruitment process? What didn't go so well?
- How did you find the consent process for WGS? Did you feel like you were given the information and support you needed to make a decision? What went well? What didn't go so well?

Expectations of WGS for CP

- When you consented for your child to have WGS, what were your expectations from WGS?
- What were your hopes for the testing? What do you think your child's hopes were?
- What concerns did you have about the testing? What concerns do you think your child had?
- What did you think the likelihood was that a genetic diagnosis would be found in your child?
- What type of impact did you think a genetic diagnosis would have on your child? On you? On your family?

Experience of waiting for, and communication of, result

- How long did it take for you to receive your child's WGS result?
- What was it like waiting for the WGS result?
- Did your child receive a genetic diagnosis? What type of result did they receive? (Positive or negative)
- How did you receive the result? (face-to-face, video, phone, letter)
- Who gave you the result?
- Did you feel you received enough information about result? Were your questions answered?
- What went well about receiving the result? What didn't go so well?

Perceived clinical impact of WGS on child and family

- Did the WGS result change your child's treatment or medical management in any way? If so, how?
- Did the WGS result give you any additional information about your child's condition? About what to expect in the future? If so, what was that?
- Did the WGS result give you more information about the chances that you or one of your child's other family members has a medical condition? If so, what was that?
- Did the WGS result provide any information about risk to future children? If so, did it have any effect on family planning?
- Overall, do you feel that the WGS result was beneficial or harmful to your child or family from a medical standpoint in any way? If so, how?

Perceived psychological impact of WGS on child and family

- How did your child react to receiving their WGS result?
- How did you and your family react to receiving the WGS result?
- Did the WGS process or result affect how your child, you, or your family was coping with your child's CP diagnosis? If so, how?
- From an emotional standpoint, what effect do you feel that having WGS for CP had on your child? What effect do you feel it had on you and your family?

For families whose child <u>did</u> receive a genetic diagnosis from WGS

- What did it mean to you to receive a diagnosis for your child?
    - Why do you think you felt that way?
- How do you think receiving a genetic diagnosis made your child feel?
    - Why do you think they felt that way?
- Do you feel that the consent process adequately prepared you for receiving a genetic diagnosis for your child? Are there any changes to this process you would recommend?
- Did the WGS result affect your child's eligibility for support services? If so, how?
- Did receiving a genetic diagnosis mean that your child or family could seek support from a group associated with that diagnosis? If so, what were the advantages and/or disadvantages of this?

For families whose child <u>did not</u> receive a genetic diagnosis

- How did you feel about not receiving a genetic diagnosis for your child with WGS?
- How do you think your child felt about not receiving a genetic diagnosis?
- Do you feel that the consent process adequately prepared you for not receiving a genetic diagnosis for your child? Are there any changes to this process you would recommend?

Advice for other families of children with CP considering WGS

- What advice do you have for other families of children with CP who are considering WGS testing?

Advice for health professionals offering WGS for children with CP

- What advice do you have for health professionals if they are offering children with CP WGS?

18.2. APPENDIX 2: Amendment History

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC committee and HRA (where required).

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	3.0	09 May 2023	Emily Li	**Study titles:** Added REC number and ISRCTN number. **Key Study contacts:** Clarified that Dr Anna Basu will be the co-investigator in Newcastle, and that that Dr Manal Issa will be the co-investigator in ESNEFT. **Summary Eligibility Criteria & Section 8.2:** Clarified that the age range for inclusion (0-16 years) is inclusive i.e. children can be included up to the age of 17. **Section 9.2**: Added that visit 1 could also occur immediately before a routine clinic appointment. **Section 9.5:** Study ID numbers will follow the format NNETxyyyzz instead of NNETxyyy_zz (removed underscore between yyy and zz). **Section 11**: Added a statement that EGLH will provide the study team with a list of variants for each proband. The list will contain no identifiable information and will be stored on the standard University of Cambridge IT network.
2	4.0	22 May 2023	Emily Li	**Study design:** Added '(up to 132)' to clarify the maximum number of biological parents that could be recruited.