Muscle connective tissue in limb development and disease

Sponsor

Name: Professor Reza Razavi, Vice President & Vice Principal (research), King's College London
Address: Room 5.31, James Clerk Maxwell Building, 57 Waterloo Road, London SE1 8WA
Telephone: 020 7848 3224
Email: reza.razavi@kcl.ac.uk

Chief Investigator

Name: Professor Malcolm Logan
Address: 5th Floor, Hodgkin Building,
Guy's Campus, King's College London, London SE1 1UL
Telephone: 020 7848 6886
Email: malcolm.logan@kcl.ac.uk

Name and address of Co-Investigators

Name: George Murphy (PhD Student)
Address: Department of Plastic Surgery, Level 7, Paul O'Gorman Building,
Great Ormond St Hospital for Children, Great Ormond St, London, WC1N 3JH
Telephone: 07714 105236
Email: georgemurphy@nhs.net

Name: Mr Branavan Sivakumar, Consultant Plastic Surgeon / Clinical Supervisor
Address: Department of Plastic Surgery, Level 7, Paul O'Gorman Building,
Great Ormond St Hospital for Children, Great Ormond St, London, WC1N 3JH
Telephone: 0207 405 9200, Ext 5222
Email: branavan.sivakumar@gosh.nhs.uk

Name: Miss Gill Smith, Consultant Plastic Surgeon / Clinical Supervisor
Address: Department of Plastic Surgery, Level 7, Paul O'Gorman Building,
Great Ormond St Hospital for Children, Great Ormond St, London, WC1N 3JH
Telephone: 0207 405 9200, Ext 5222
Email: Gill.Smith@gosh.nhs.uk

CONTENTS

Study Synopsis

Title of clinical study	Muscle connective tissue in limb development and disease
Sponsor name	King's College London
Chief Investigator	Professor Malcolm Logan
REC number	**London Hampstead – 15/LO/2085**
Medical condition or disease under investigation	Radial dysplasia
Purpose of clinical trial	The objective of this work is to understand how the disruption of the muscle connective tissue contributes to the limb soft-tissue defects in radial dysplasia. In parallel, we will investigate the role of muscle connective tissue in normal limb development.
Primary objective	The primary outcome measure will be the characterization of differences in muscle connective tissue and its derivatives between normal controls and patients with radial dysplasia. This will include tissue histology, and gene/protein expression.
Secondary objective (s)	The secondary outcome measures will be: • The identification of novel biomarkers for human muscle connective tissue derivatives, which may potentially serve as diagnostic or prognostic tools • The potential identification of a stem-cell-like or progenitor pool for muscle connective tissue in mature soft tissues.
Trial Design	Basic laboratory research, with a cross-sectional case-control design for sample collection.
Endpoints	N/A
Sample Size	A pragmatic sample of **30** radial dysplasia patients and a minimum of 15 control samples, based on the departmental patient caseload.
Summary of eligibility criteria	Inclusion: • The patient is attending for planned reconstructive surgery for radial dysplasia, *or* • The patient is attending for reconstructive surgery after hand trauma (control patients), *or* • The patient is attending for other elective hand surgery (control patients). Exclusion: • Patients with extensive previous scarring to their forearm and hand. • Patients with a significant pathological skin or soft tissue lesion at the donor site.
Version and date of final protocol	Version 5.3, 09 November 2015
Version and date of protocol amendments	**Version 6.0, 02 July 2018**

1. Background & Rationale

Brief description

The objective of this work is to understand the origins of the soft tissue defects in a disabling, disfiguring limb anomaly called radial dysplasia, and what mechanisms of normal limb development have been disrupted in these patients to produce their phenotype. We aim to use this knowledge to improve their treatment. Currently, despite sophisticated surgical treatment, the limb malformation the child is born with typically recurs as they grow: it is thus an 'unsolved problem'.

We have previously shown, in an experimental mouse model of radial dysplasia, the underlying soft tissue problem is a change in the muscle connective tissue and its derivatives, causing abnormal soft-tissue patterning. We wish to expand this work into our patient population by comparing samples of post-natal muscle connective tissue derivatives from radial dysplasia patients with control samples.

We will ask patients undergoing corrective hand surgery to let us take small tissue samples during their planned operations. We will also ask patients having other forms of hand surgery, such as surgery for hand injuries, to let us take similar samples for comparison. In either case, their scar and peri-operative treatment will be unchanged. We will examine the tissue samples in our laboratory to look for changes in tissue architecture, cellular composition, cell signalling, and how they behave when grown in culture. We will also make attempts to derive cell lines from biopsy samples to use in further studies, exploring cellular phenotype and functional capacity.

Simultaneously, we will look at the long-term surgical outcomes and the range of genetic changes in our patient population. Eligible patients will be referred for consideration in the (separate) 100,000 genome project; this should further expand our knowledge of the genetic changes underlying limb anomalies. The ability to combine data on genotype, long-term phenotype and soft tissue changes will give a comprehensive overview of the condition. We expect this to lead to a better understanding of patient subgroups, and to provide a rational basis for improved treatment approaches.

Population to be studied

- **Patients of any age** with a clinical diagnosis of radial dysplasia, undergoing planned reconstructive surgery.
- **Patients of any age**, undergoing reconstructive surgery for hand trauma (control patients).

Background

Limb development is controlled by a tightly regulated cascade of events, and disruption can lead to limb birth defects. One such anomaly is radial dysplasia. Affected children (1:50,000 births) have a dramatically short and radially deviated forearm, with a stiff elbow and characteristic hypoplastic or absent thumb[1]. All forearm tissue types are involved, and most affected children fall at the severe end of the disease spectrum. Children can be affected following spontaneous mutations, teratogenic drugs or as part of a syndrome, such as Holt-Oram, VACTERL or Fanconi syndrome.

Most specialist centres begin treatment with soft tissue distraction, then perform a wrist stabilisation procedure, including tendon transfers and the creation of a stable forearm-wrist interface[2]. An alternative technique involves microvascular transfer of a joint from the child's toe to wrist, providing a buttress to support their hand[3]. However, despite the sophistication of these techniques, children still commonly suffer recurrent radial deviation and impaired forearm growth[1]. Radial dysplasia is, therefore, an 'unsolved problem', with frequently debilitating lifelong consequences[1].

The key to improved treatment lies in understanding the mechanisms of limb development, and thus the underlying cause of limb birth defects. One essential, but hitherto under-studied part of the limb

developmental cascade is muscle connective tissue (MCT). MCT plays a critical, instructive role in the formation of individual muscle bundles during embryonic development, 'patterning' the myocytes to develop into the familiar individual muscles[4-6]. MCT cells ultimately produce the epi, peri and endomysium, and contribute to other limb fascial layers[7]. Defective MCT in animal models leads to the soft-tissue deformities seen in radial dysplasia[7,8] and potentially contributes to recurrent limb deviation after surgery. More broadly, MCT and its derivatives are implicated in muscle homeostasis and repair[7].

Based on our mouse model data (below), we predict that changes in the MCT and its post-natal derivatives are a causative factor in both the initial limb anomaly and to recurrent radial deviation. Our initial long-term follow-up series suggests there are distinct patient subgroups, defined by their response to soft-tissue distraction, who benefit from different surgical approaches[9]. We further predict that differences in the MCT derivatives will correlate with these clinical subgroups, informing the optimal treatment approach and outcome. Furthermore, understanding the normal and abnormal soft tissue development of the upper limb will advance our ability to perform tissue engineering, which may improve our ability to control and direct muscle formation.

This proposal aims for the first time properly to describe MCT abnormalities in radial dysplasia. We aim to develop our long-term treatment outcome study for radial dysplasia, and aim to confirm our preliminary finding of patient subgroups that respond better to either surgical centralisation or radialisation of the ulna on the basis of their soft tissues, and thus allow rational treatment selection. The knowledge gained about normal limb development could also have great potential for application in tissue engineering.

Background data:

- We have previously demonstrated the importance of MCT both for normal limb soft tissue development and that disruption of MCT in a mouse model of Holt-Oram syndrome can explain the soft tissue dysplasia associated with this syndrome[5,8]. In humans, *Tbx5* mutations cause Holt-Oram syndrome, a congenital form of radial dysplasia. We demonstrated that the soft tissue abnormalities in Holt-Oram are explained by disruption of *Tbx5* function in MCT, occurring at a distinct time and location from the origins of the skeletal defects. Further, selective genetic ablation of limb MCT cells leads to an even more dramatic disruption of muscle pattern.

- We have used genetic tools in mice to fluorescently label and purify MCT using fluorescent cell sorting (FACS). We performed a whole genome transcriptome screen, yielding a list of genes expressed in MCT. This list has been analysed using bioinformatics tools. Some are markers expressed at the cell surface, offering potential for use in live cell sorting. Of particular functional significance, several genes identified code for members of the small leucine-rich proteoglycan (SLRP) family. These are a well characterised family of secreted proteoglycans that are known to organise the extracellular matrix, regulate collagen fibrillogenesis in both tendon[10] and corneal formation[11], and modify cell-cell signalling across the extracellular matrix[11]. Abnormal SLRP expression is known to cause disordered extracellular matrix and clinical pathology[11].

- A cohort of MCT cells is active during embryogenesis; however it is not known if these cells are retained in later life and 'reactivated' during tissue homeostasis, repair or regeneration. Such a population may be analogous to muscle 'satellite cells'. We plan to analyse tissue biopsies to look for evidence of an equivalent population of MCT cells.

Summary of risks and benefits

The risks following sample collection will not differ from those of routine reconstructive hand surgery. There is no change to the post-operative functional outcome, pain, wound healing or cosmesis. Patients will receive standard peri-operative care, pain relief, regional or local anaesthesia, splinting and physiotherapy.

We do not expect patients to benefit directly from participating in the study; this is an altruistic donation. However, we do expect that this research will lead to a better understanding of radial dysplasia, and possibly to new treatments in future, which there is a limited chance these patients may benefit from.

Ethical conduct statement

- The study will be conducted in compliance with the principles of the World Medical Association Declaration of Helsinki, as amended at the 64th WMA General Assembly, Fortaleza, Brazil, October 2013 (http://www.wma.net/en/30publications/10policies/b3/)
- The study will be conducted in accordance with the standards laid out in "Good Clinical Practice", as promulgated by the UK National Institute for Health Research (NIHR).
- The study will be submitted for ethical review to the London Bloomsbury research ethics committee (REC), Manchester HRA Centre, 3rd Floor, Barlow House, 4 Minshull Street, Manchester, M1 3DZ. Any subsequent protocol amendments will be supplied to the REC, along with progress reports and the final study report.

References

1. *Goldfarb CA, Klepps SJ, Dailey LA, Manske PR. Functional outcome after centralization for radius dysplasia. The Journal of hand surgery. 2002;27(1):118-124.*
2. *Buck-Gramcko D. Radialization as a new treatment for radial club hand. The Journal of hand surgery. 1985;10(6 Pt 2):964-968.*
3. *Vilkki SK. Distraction and microvascular epiphysis transfer for radial club hand. Journal of hand surgery. 1998;23(4):445-452.*
4. *Kardon G, Harfe BD, Tabin CJ. A Tcf4-positive mesodermal population provides a prepattern for vertebrate limb muscle patterning. Developmental cell. 2003;5(6):937-944.*
5. *Hasson P, DeLaurier A, Bennett M, et al. Tbx4 and tbx5 acting in connective tissue are required for limb muscle and tendon patterning. Developmental cell. 2010;18(1):148-156.*
6. *Mathew SJ, Hansen JM, Merrell AJ, et al. Connective tissue fibroblasts and Tcf4 regulate myogenesis. Development. 2011;138(2):371-384.*
7. *Hasson P. "Soft" tissue patterning: muscles and tendons of the limb take their form. Developmental dynamics : an official publication of the American Association of Anatomists. 2011;240(5):1100-1107.*
8. *Logan Laboratory. Besse L, Manuscript in preparation. 2015.*
9. *Kampfen A SB, Miller C, Jessop N, Smith G, Smith P. Long term outcome of radial club hand correction. 10th World Symposium on Congenital Malformations of the Hand and Upper Limb; 7/5/2015, 2015; Rotterdam, NL.*
10. *Ezura Y, Chakravarti S, Oldberg A, Chervoneva I, Birk DE. Differential expression of lumican and fibromodulin regulate collagen fibrillogenesis in developing mouse tendons. The Journal of cell biology. 2000;151(4):779-788.*
11. *Chen S, Birk DE. The regulatory roles of small leucine-rich proteoglycans in extracellular matrix assembly. The FEBS journal. 2013;280(10):2120-2137.*

2 Study Objectives, Design and Statistics

2.1. Study Objectives

Aims

1. To clarify the existence of treatment subgroups by expanding our long-term follow-up database for radial dysplasia, and to correlate these with the patient's genotype and soft tissue changes.

2. To characterise markers of MCT derivatives in mouse tissue samples and confirm these as MCT derivative markers in human tissue.

3. To characterise the extent and nature of disruption to the MCT derivatives in patients with radial dysplasia, and;
    a. establish it as a significant causative feature of the clinical presentation.
    b. establish whether MCT status can be diagnostic / prognostic.

4. To look for a 'satellite cell / stem cell' like population in mature fascia.
    a. If this population is identified, to determine whether this population of cells diminishes with age.
    b. If this population of cells cannot be identified in mature fascia, to determine when the population of 'embryonic' MCT cells is lost.

5. To expand our knowledge of the genetic mutations underlying radial dysplasia.

Objectives

1) The primary objective will be the characterization of differences in muscle connective tissue and its derivative between normal controls and patients with radial dysplasia. This will include tissue histology, and gene/protein expression.
2) The secondary objective will be:
    a) the identification of novel biomarkers for human muscle connective tissue, which may potentially serve as diagnostic or prognostic tools, and
    b) the potential identification of a stem-cell-like or progenitor pool for muscle connective tissue in mature soft tissues.

2.2 Study Design

All patients attending Great Ormond Street Hospital for reconstructive hand surgery for radial dysplasia, under the care of Mr Bran Sivakumar or Miss Gill Smith, will be considered for recruitment after being fully informed about the research, and having given informed (parental) consent. Interviews with patients and their families for recruitment will take place as part of or alongside a routine clinical visit by the patient to the hospital. No separate, specific research interviews will be required.

Recruited participants will have 2-3 small tissue samples taken during 1-2 of their planned reconstructive surgical procedures, whilst under general anaesthesia in the operating theatre. Samples will be taken by scalpel or scissors, by the operating surgeon, from within the surgical site in the forearm and hand. The skin incision and deep dissection will have to be made as part of the normal course of reconstructive surgery, regardless of participation in this study.

The samples will be ellipses of tissue measuring no more than 5 x 10 mm, or less at the discretion of the operating surgeon. Samples may include muscle, muscle connective tissue, tendon, fat and skin, or a combination of the above. Although these samples would not be taken if the patient were not involved in research, their removal will have no impact on the patient's post-operative hand function, recovery time or scar. We may also collect any tissue routinely excised as part of the reconstructive procedure, which would otherwise be discarded.

Age and sex-matched control samples will be collected in the same fashion by surgical members of the team from patients undergoing surgery for hand trauma or other elective hand conditions. These samples may also include muscle, muscle connective tissue, tendon, fat and skin, or a combination of the above. Once again, although these samples would not be taken if the patient were not involved in research, their removal will have no impact on the patient's post-operative hand function, recovery time or scar. As above, we may also collect any tissue routinely excised as part of the reconstructive procedure, which would otherwise be discarded.

In both cases, patient follow-up after surgery will be exactly the same as the routine follow-up for their condition, with the same dressing changes, physiotherapy, hand splinting regime and clinic visits as normal for their condition.

Sample storage

Once collected, samples will be placed in sterile plastic containers. All samples will be anonymised by allocation of a sequential patient identification number, which can only be linked to patient identity by members of the surgical team. Containers will be labelled with the identification number and the nature of the biopsy (eg "patient 4, muscle connective tissue, distal radial"). In accordance with a material transfer agreement between the hospital and King's College London, the samples will then be taken from the operating theatre by George Murphy (a member of both the laboratory and clinical teams), and conveyed by **registered courier or** private transport (on foot or by private bicycle) to the Logan laboratory, room HB 5.30w, at Guy's campus, King's College London. Samples will be processed immediately and stored in a **tissue culture incubator**, refrigerator, liquid nitrogen or a -80C freezer. Anonymised data about the patient's clinical condition may be shared with the laboratory team by the surgical team, using the same identification number, but no patient identifiable data will be shared outside the immediate clinical team.

Laboratory methods

To explore the hypothesis that abnormal muscle connective tissue and its derivatives are responsible for the soft tissue deformities in radial dysplasia, we will begin by examining the tissue architecture with histological stains such as von Kossa, van Gieson (for collagens and connective tissue) and haematoxylin and eosin. We predict that the extracellular matrix, in particular the arrangement of collagen fibres, will be significantly disordered in radial dysplasia patients. This can be compared between radial dysplasia patients and controls, and in the same patient before and after soft tissue distraction. We will develop this further using immunohistochemical assays and confocal microscopy to develop a three-dimensional picture of the MCT and the extracellular matrix, and the changes in small leucine-rich proteoglycan expression. We will also use in-situ hybridisation and immunohistochemistry to explore the role of muscle connective tissue derivatives, and to look for the presence or absence of muscle connective tissue derivatives displaying a satellite cell or stem cell like phenotype.

To confirm the relationship between the MCT derivatives and the extracellular matrix, the gene expression pattern in the tissue biopsies will be interrogated using a combination of antisense RNA probes and/or antibodies using in-situ hybridisation and immunohistochemical assays. The host laboratory have substantial experience using these techniques on muscle connective tissue within the mouse embryonic limb.

We will then explore the ability of the MCT cells isolated to grow in culture. Magnetic activated cell sorting (MACS) will be used to purify muscle connective tissue based on cell surface markers. The isolated cells will be cultured and co-cultured in-vitro, as we have successfully done in mouse tissue.

All of the above techniques are well established in the laboratory for mouse tissue; this will extend the work to human tissue.

2.3 Study Flowchart

Radial dysplasia patients	Outpatient clinic visit	Pre-assessment clinic visit	Day of applying distractor (First surgery)	Day of wrist stabilisation (Second surgery)
Confirm eligibility.	X	X		
Patient information and informed consent.	X	X		
Refer for genetic review (includes consideration, where appropriate, for recruitment to NIHR biobank)	X			
Collect intra-operative tissue samples.			X	X

Control patients	Outpatient / trauma clinic visit	Accident & Emergency visit	Day of Surgery
Confirm eligibility.	X	X	
Patient information and informed consent.	X	X	
Collect intra-operative tissue samples.			X

2.4 Trial Statistics

2.4.1 Sample Size

Our primary recruitment target is **45** patients (**30** radial dysplasia patients, 15 controls) within 6 years.

As this study is entirely basic laboratory research, we have not conducted a power calculation.

The Hand surgery department at Great Ormond Street sees approximately 10 new cases of radial dysplasia per annum; we aim to recruit **30** cases over the **six** years of the study, plus 15 control patients. This represents a 50% case recruitment rate, which is conservative by historical standards, and we feel is very achievable.

For control samples, **15 patients should be achievable, as we are working with three large paediatric hand trauma services (Oxford University Hospitals, Chelsea and Westminster & the Royal Free Hospital)**.

The sample size will provide sufficient material to produce a robust comparative analysis data set that will

reveal any differences in connective tissue morphology/integrity in samples from patients with or without radial dysplasia.

The sample size will also provide sufficient material to test and identify novel markers of muscle connective tissue.

2.4.2 Analysis

Methods of analysis
We will undertake a comparative analysis of tissue samples from radial dysplasia patients (pre and post soft tissue distraction) and healthy controls. This will use histological, immunohistochemical and transcriptomic analysis of gene expression. Initially qualitative differences will be described.

Further quantitative analysis of immunofluorescently labelled samples or of transcript levels will be performed using image processing software and/or RNAseq/QRT-PCR, and then subjected to appropriate statistical analysis (eg paired t-test, ANOVA, R package and/or Avadis platform). The host laboratory are familiar with these techniques and their statististical analysis.

3 Selection and Withdrawal of Subjects

3.1 Recruitment and Informed consent

Identification of patients
Recruitment will take place from the caseload of patients referred to the hand surgery team at Great Ormond St Hospital for treatment of radial dysplasia. All patients referred will be considered for inclusion.

Recruitment of control patients will take place from the caseload of patients referred to the hand surgery teams at **Oxford University Hospitals**, Chelsea & Westminster and the Royal Free hospitals for treatment of hand trauma, and other elective hand surgery. All patients referred will be considered for inclusion.

Participants will be identified by three routes:

1) Screening of new and existing congenital hand anomaly patients as they attend their routine clinic appointments at Great Ormond St. This will be conducted by their direct care team lead (Mr Bran Sivakumar or Miss Gill Smith).
2) The families of patients already booked for reconstructive hand surgery at the start of the study period (ie those on the waiting list) will be contacted by telephone by a member of the surgical team to discuss the study, and information sent by post.
3) Patients and their families attending the **adult and** paediatric hand trauma clinics at **Oxford University Hospitals**, Chelsea & Westminster and the Royal Free hospitals will be screened by a member of the surgical team.
Patients will not be recruited on the day of surgery, as this would not give the patient and their family sufficient time to consider their participation, especially under the stress of an imminent operation.

Participant recruitment; first approach
Great Ormond St hand surgery team, lead by Mr Bran Sivakumar and Miss Gill Smith, or:
Hand surgery team at Chelsea & Westminster Hospital, lead by Miss Gill Smith, or:
Hand surgery team at the Royal Free Hospital, lead by Mr Bran Sivakumar, or:
Hand surgery team at Oxford University Hospitals, lead by Miss Lucy Cogswell.
Informed consent?

Yes. **Informed consent will be obtained from the patient if over 16, or a parent or person with parental responsibility for any child under 16**. Children mature enough to do so will additionally be offered the chance to assent. This will be in addition to the normal consent procedure for their surgery.

Time for consideration
A minimum of 24 hours, if patients are admitted in advance of their surgery; the majority of patients will have at least 2 weeks to decide.

Care for patients with English as an additional language
Interpreters are employed on-site at Great Ormond St, and all **four** hospitals have access to telephone interpreters via 'LanguageLine' or similar.
Patient information sheets will involve age-appropriate diagrams for children with limited written or spoken comprehension.

Recruitment will run until **1 February 2022**. Laboratory experiments will run concurrently with this and continue until all experiments have been completed.

3.2 Inclusion Criteria

Study Group

- Patients with a clinical diagnosis of radial dysplasia, requiring **reconstructive surgery.**
- **Any age** (in nearly all cases, this surgery occurs over the age of 1).
- Either sex.
- Informed (parental) consent to participate.

Control Group

- Patients with an injury requiring **reconstructive surgery.**
- **Any age**.
- Either sex.
- Informed (parental) consent to participate.

3.3 Exclusion Criteria

Study Group

- Patients with a clinical diagnosis of radial dysplasia, but not requiring **reconstructive surgery**.
- Patients with a diagnosis other than radial dysplasia.
- Patients with extensive previous scarring to their forearm and hand.
- Patients with a significant pathological skin or soft tissue lesion at the donor site.

Control Group

- Patients with extensive previous scarring to their forearm and hand.
- Patients with a significant pathological skin or soft tissue lesion at the donor site.

3.4 Withdrawal of Subjects

If a patient / their family loose capacity to consent, we will retain and use any data and tissue already collected. No further data or tissue will be collected or any other research procedures carried out on or in relation to the participant.

However, if a patient / their family withdraw their consent, we will withdraw all their identifiable tissue and data from the study.

This will be made clear during the consent process, and on the written consent forms.

In the extremely unlikely event of an adverse event related to the study, patients and their family would continue to be offered the appropriate care for their condition by the clinical team. They would also be offered a full description of the event, and its possible consequences, in line with the duty of candour.

3.5 Recruitment of Children

Patients between the ages of 0 and 18 are treated at Great Ormond Street Hospital. The majority are operated on between the age of 1 and 5. As our tissue biopsies are tied to their planned surgery, this will be the age range we recruit.

Age and sex matched control patients will be recruited, where they are undergoing planned hand surgery, either for a fully elective condition or for hand trauma. No patients will be recruited on the day of surgery.

Consent
Informed parental consent will be sought from the person with parental responsibility, and the child if 'Gillick/Frazer competent' during their outpatient visit, prior to their child's admission. Consent is then confirmed with the parents on the day before or the day of surgery.

Participants recruited to this study will have been informed about the research at least 24 hours prior to surgery, at either an outpatient clinic, telephone recruitment, pre-operative planning clinic or inpatient admission the day before surgery. Confirmation of consent for research participation will take place during the confirmation of surgical consent on the day before or day of surgery.

Tailoring of information to child's understanding:
Separate participant information sheets will be provided for parents and children.

The main children's information sheet will contain simple language and diagrams appropriate for a 5-10 year old age group. This will emphasise that there will be no pain or change in their outcome associated with study participation.

For children younger than 5, or where appropriate when over 5, we will inform them at an appropriate level of understanding, with their parents help.

4 Security and Confidentiality

4.1 Data Security

Patient medical records and referral details will be screened by members of the surgical care team during their routine clinical contact. No access to these records will be available to anyone outside the routine surgical care team. All records will remain in their normal clinical setting. Patient-identifiable information will be restricted to the usual computer systems and physical records present in the **four** hospitals

concerned, and transmission between hospitals will only occur between secure nhs.net email addresses, for the purpose of maintaining the study database at Great Ormond St Hospital.

Data management
In addition to data in the routine medical records, a database containing the patient study identification number, and patient identifiable information, will be maintained on NHS computers at Great Ormond Street Hospital. Physical copies of the research consent forms will be kept in the Plastic Surgery office in Great Ormond Street, which is secured behind three layers of access-controlled doors.

Communication between sites will be from and to secure nhs.net email accounts.

Patient clinical characteristics, but not patient identifiable data, will be shared with the laboratory team, and maintained on a database at King's College London (Guy's Hospital Campus); this is secured behind a manned porter's desk and access-control doors.

Data confidentiality
Only the direct surgical care team will have access to patient-identifiable data during the study. This will take place during routine clinical contact on NHS premises.

All samples will be labelled with an anonymised code, which can only be linked to patient identifiable data by the surgical care team. This will consist of a sequentially allocated patient identification number. Containers will be labelled with the identification number and the nature of the biopsy (eg “patient 4, muscle connective tissue, distal radial”). The database linking the patient identification number to their identifiable data will be maintained on NHS computers at Great Ormond Street.

Data analysis / storage during project
Clinical data will be analysed at the **four** hospital sites, mostly at Great Ormond St, by George Murphy (a Plastic surgeon holding an **(honorary)** clinical contract at each site). Transfer of data between NHS sites will occur electronically from and to secure NHS.Net email accounts. Clinical data will remain under the control of the clinical care team, in accordance with the policy at each hospital. The lead Consultant for each site (Mr Sivakumar, Miss Smith **or Miss Cogswell**) will act as custodian for the clinical data generated; this will not be shared outside the clinical team.

Laboratory samples will be analysed at Professor Logan's Laboratory at Guy's Campus, King's College London. **Analysis will be performed by George Murphy, Professor Logan and the other members of his laboratory.** Professor Logan will have control of and act as custodian for the laboratory data generated by the study.

4.2 Data Archival

Routine clinical follow-up for congenital hand surgery patients occurs until skeletal maturity, at about age 18. Data will be stored as part of the patient record, and kept in accordance with **each hospital’s** routine record storage and retention policy.

Patient-identifiable data will be stored electronically on Great Ormond St NHS trust computers. Access will be password restricted to members of the hand surgery team. The trust complies with all current data protection requirements, and can reasonably be expected to maintain its infrastructure and policies to remain compliant with any future regulations. The trust mainframe is a well-maintained, firewall and password protected and securely backed-up system.

Anonymised data will be stored physically within Professor Logan's Laboratory, and electronically within the system of King's College London. This again is a well-maintained, firewall and password protected and securely backed-up system. Access will be restricted to members of Professor Logan's Laboratory.

4.3 Tissue Security

Samples will be stored in the Logan laboratory, room HB 5.30w, 5th Floor, Hodgkin building at Guy's Campus. Access to the laboratory is restricted by secure ID card through access control doors, plus the presence of a security team at the front desk. Only members of Professor Logan's laboratory will have access to these samples.

Within the laboratory, samples will be stored in a **tissue culture incubator**, refrigerator, -80 degree freezer, or in liquid nitrogen, as appropriate.

At the end of the project, samples will be retained by the research team pending ethical approval for use in another project. We would expect as a direct consequence of this research to perform other research using the same tissue and cells. We have a section in the research consent form that describes this eventuality. Once any further research directions become clearer, we will submit new applications for ethical review.

5 Assessment of Safety

5.1 Specification, Timing and Recording of Safety Parameters.

Patients will undergo routine intra-operative and peri-operative monitoring, as per the normal practice of the anaesthetic team involved. No additional monitoring beyond standard care is required.

5.2 Procedures for Recording and Reporting Adverse Events

Adverse events will be reported through the relevant hospital incident reporting process, and also reported (as linked anonymised reports) to the site Principal investigator.

6. Direct Access to Source Data and Documents

Where required by the REC, or by an appropriate regulatory body, access will be granted to laboratory data. Regulatory access to patient records will be determined by the relevant hospital policies.

7. Ethics & Regulatory Approvals

- The study will be conducted in compliance with the principles of the World Medical Association Declaration of Helsinki, as amended at the 64th WMA General Assembly, Fortaleza, Brazil, October 2013 (http://www.wma.net/en/30publications/10policies/b3/)
- The study will be conducted in accordance with the standards laid out in “Good Clinical Practice”, as promulgated by the UK National Institute for Health Research (NIHR).
- The study will be submitted for ethical review to the London **Hampstead** research ethics committee (REC), Manchester HRA Centre, 3rd Floor, Barlow House, 4 Minshull Street, Manchester, M1 3DZ. Any subsequent protocol amendments will be supplied to the REC, along with progress reports and the final study report.

8. Quality Assurance, Data Handling, Publication Policy and Finance

- The project will be overseen by Professor Logan, who will be responsible for quality control.
- The project received formal review by KCL during the process of agreeing sponsorship, and also full external peer-review by the Great Ormond St Clinical Research Adoptions Committee. Additionally, informal review was undertaken during development within the research team at the Randall and Great Ormond St.
- Clinical data will only be accessible to clinical staff, and will be the ultimate responsibility of the Consultant caring for the patient (Mr Bran Sivakumar or Miss Gill Smith). The clinical database will be maintained at Great Ormond St Hospital by George Murphy, with communication between hospitals restricted to nhs.net email accounts.
- Laboratory data will be stored in the Logan laboratory at Guy's Campus, behind access control doors, with computerised records on the King's College London mainframe.
- Data will be published in peer-reviewed journals, most likely as a series of articles.
- The Logan laboratory is supported by an MRC **research grant**.
  George Murphy is supported by a PhD grant from the British Society for Surgery of the Hand.
- This study has been registered with Clinicaltrials.gov (**NCT02611089**).

9. Signatures

_____ _____

Chief Investigator – Malcolm Logan Date

_____ _____

Student – George Murphy Date