NephroS

National Study of Nephrotic Syndrome (NephroS) Protocol

Version 7

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1. INTRODUCTION

Idiopathic Nephrotic Syndrome can broadly be classified as Steroid Sensitive (SSNS), or Steroid Resistant (SRNS). A significant proportion of SRNS patients demonstrate steroid sensitivity early in their disease course.

Steroid Resistant Nephrotic Syndrome (SRNS) and principally Focal Segmental GlomeruloSclerosis (FSGS) is rare in childhood and adulthood. However, it produces a significant amount of morbidity. There are approximately 270 children in the UK with SRNS/FSGS currently and 10% of those are on the end stage renal failure programme. Approximately 12 children, and an undetermined number of adults with SRNS/FSGS undergo transplantation per year in the UK. The literature reports that 30-50% of patients will have recurrence of disease post transplantation and up to 100% following second or third transplants. There is a heavy burden on the patient and NHS services.

It has been difficult to further our understanding of the disease because of the small numbers of patients, however the development of the Rare Kidney Disease Registry (RaDaR) in the UK will allow for identification of a large cohort of children and adults, thus enabling meaningful research.

1.1 Study background and rationale

The glomerulus is the elemental unit of renal filtration. Its unique role is dependent on an intact structure and function of the podocyte, the central cell of the glomerular filtration barrier. Damage to podocyte integrity is a consistent feature of all acquired Nephrotic Syndromes, one of the most severe forms of these being Focal Segmental GlomeruloSclerosis (FSGS). An ongoing enigma in FSGS is the involvement of putative circulating factors in its pathogenesis [1], and now a link has begun to emerge between plasma 'factors' and the podocyte [2]. However, no such factor has yet been discovered, and *in vitro* permeability assays to study and predict disease activity have so far proven inconsistent[3]. A particularly dramatic feature of acquired FSGS is the rapid recurrence of the disease in a large proportion of renal transplants, and it is likely that this subset of patients has a unique disease mechanism based on circulating plasma abnormalities. The only

current treatment for these patients is intensive plasma exchange and heavy immunosuppression, which can lead to major morbidity and death[4]. Now, advances in our molecular understanding of podocyte biology have begun to unravel the mechanisms underlying Nephrotic Syndromes. Initially, the molecules associated with early onset congenital nephrotic syndromes, nephrin[5] and podocin[6], were associated with the specialised podocyte cell-cell junction known as the slit diaphragm, which links to the actin cytoskeleton. Knockout of CD2AP[7], an adaptor protein that links nephrin/podocin to the actin cytoskeleton, results in congenital Nephrotic Syndrome in a mouse model, and mutations in α-actinin-4, an actin binding molecule, cause adult onset FSGS[8]. Thus, in human diseases to date, slit-diaphragm/actin-associated proteins appear to be important in maintaining podocyte integrity.

TRPC6 (transient receptor potential canonical channel –6) is a Ca^{2+}-permeable non-selective cation channel, expressed in podocytes, which has minimal basal activity and is activated via transduction cascades initiated by G protein-coupled receptors or receptor tyrosine kinases[9, 10]. Recently, novel pedigrees of patients have been reported, with mutations described resulting in inappropriate activation of the TRPC6 channel, leading to adult-onset FSGS[11, 12]. This provides the key insight that aberrant basal activation of TRPC6 results in a form of FSGS and implies that a tight control of podocyte cation/Ca^{2+} currents is essential for the integrity of the slit-diaphragm.

The research group have found, using conditionally immortalised human podocyte cell lines (ciPod) derived from normal kidneys[13] and from a child with a Fin Major (ND) nephrin mutation[2], that in ciPod, nephrin is necessary for constitutive direct suppression of TRPC6 activity, and surprisingly that this suppression is present only in the presence of normal human plasma (HPL). They have also found that HPL acts via RhoA, a cytoskeletal regulator, to correctly localise nephrin at the plasma membrane (unpublished data).

Interestingly, and supporting the previous observation, nephrotic plasma from FSGS relapse patients induces TRPC6 activation, compared to remission plasma, and reverses the nephrin localisation, and this sequence of events can be suppressed by RhoA inhibition. Therefore

they speculate that distinct constituents are present in normal human plasma that are essential for suppression of TRPC6 activity, and these are absent or inhibited in the nephrotic state, suggesting their loss is linked to the pathogenesis of the disease. This indicates for the first time a functional link between acquired FSGS and slit diaphragm protein signalling.

1.2 Study Development

This study was set up in January 2010 to investigate Steroid Resistant Nephrotic Syndrome in children. Due to the success of the project, in January 2015, the research study expanded to include both adult and child patients with Idiopathic Nephrotic Syndrome. Due to the increased eligibility criteria the study was re-named NephroS (The National Study of Nephrotic Syndrome). In addition, a collaboration was set up with the NIHR BioResource – Rare Diseases study offering patients the opportunity to have their whole genome sequenced. More recently, the Research Team have been successful in obtaining additional funding to widen the study further at a select number of centres, as part of the NURTuRE - National Unified Renal Translational Research Enterprise.

NURTuRE is a collaboration between several independent investigators, Kidney Research UK and commercial companies engaged in the development of new treatments for Nephrotic Syndrome. The partnership aims to maximise the scientific value of the samples and data collected by aiming to improve the understanding of the causes of Nephrotic Syndrome and thereby helping to stratify patients according to clinical characteristics to provide them with the best treatment possible.

NURTuRE is starting with two disease areas – Nephrotic Syndrome (as described in this protocol) and the NURTuRE-Chronic Kidney Disease Study (NURTuRE-CKD, IRAS project ID 211479, sponsored by the University of Nottingham). NURTuRE represents an important development in the progress of renal research and will act as a springboard for similar developments into other aspects of kidney disease.

In order to start this NURTuRE development, we will have two study site types:Study Centre type A: Will continue with the NephroS study as before.
Study Centre type B: Will be part of the NURTuRE development. Some samples will be made available to university researchers and NURTuRE commercial partners (and people working on their behalf) and some samples centrally stored for future use. A select number of centres will be offered the opportunity to become a Study Type B Centre (NURTuRE). Some of these centres will already be recruiting to NephroS, if so, samples previously collected for patients at these centres will remain stored at the University of Bristol as before. Future samples collected from these centres will be made available to NURTuRE collaborators or biobanked for future use. If a participating Study Type B centre is unable to recruit successfully, it will be withdrawn from the NURTuRE part of the study (reverting to a Study Type A centre or withdrawn from the study) and will be replaced by another centre. Participants already recruited from a centre that withdraws in this way will remain in the study.
The Chief Investigator will have overall responsibility of the study and will oversee study management.

For the NURTuRE side of the project, a Study Management Committee (SMC) will support and coordinate the delivery of the study. The SMC will meet regularly by teleconference with face to face meetings as required. There will also be a Joint Steering Committee (JSC) which will provide oversight of the study and will review progress against the objectives and milestones. For samples biobanked, top level oversight will be provided by a Strategic Oversight and Access Committee (SOAC). The SOAC will provide governance and oversight of the biobank resource and will appoint and oversee an Independent Biosample Access Committee that will consider and approve applications for access to specimens in the biorepository from independent investigators who wish to make use of the samples.

Below summarises the activities that will take place at each Site Type (A or B) (Table 1). Detailed sample transfer is documented in Section 5.5.

	Site Type A – NephroS (continuing as before)	Site Type B – NURTuRE-NephroS
Consenting and collecting samples	Patients consented and samples collected.	Patients consented and samples collected.
Processing of Samples	No processing of samples on site - samples are sent direct to the University of Bristol	Samples are processed, aliquoted and stored at site until collected via courier approximately every 4months and sent to the NIHR National Biosample Centre (blood and urine). Biopsy slides and blocks are sent to the Biorepository at the University of Birmingham. Plasma Exchange Bags are sent directly to the University of Bristol.
Use of samples	Samples will be stored and analysed at the University of Bristol. Research may include the participation of other academic researchers/commercial companies. Upon further patient consent, some samples will be shared with BioResource-Rare Diseases for whole genome sequencing.	Samples will be available to NephroS researchers and commercial companies who are part of the NURTuRE consortium. Some samples will be biobanked and stored for future studies. Access to these stored samples will be via an Independent Access Committee.

Table 1: Site type A and B Local activities

1.3 Collection of samples

Utilising the infrastructure created by the National Rare Disease Registry (RaDaR), it will be possible to identify all adults and children with Nephrotic Syndrome (NS) in the UK on an ongoing basis. The structure is a web-page linked to via the RaDaR homepage (nww.radar.nhs.uk); approved by the South West - Central Bristol Research Ethics Committee,

reference 09/H0106/72,which all UK Nephrologists and research teams can access upon request from the RaDaR research team. Returns to this site are requested for any NS patient diagnosed at initial consultation and for future follow-up including at the time of transplantation, and six monthly thereafter to follow the course of the disease, with detailed data fields to be captured. We are interested in any hospital admissions and other medical problems that can occur in people with NS. Patients may be asked questions in routine visits regarding previous illness, past medications used and vaccination status. Details of medical history will be verified by inspection of hospital records. For future data collection, use of the NHS Information Centre is commonly used to keep in touch with health status in this kind of research study.

Biological samples will be taken at routine appointments wherever possible, however if this is not feasible, patients may be asked to visit the hospital on another occasion, or asked if they are happy to have blood taken solely for research purposes e.g. if attending a research study visit to consent.

For bloods taken at routine appointments, the following anthropomorphic/laboratory tests should be performed as part of routine clinical care (non-fasting results acceptable):

- Weight
- Height
- Blood pressure (ideally manual reading)
- Urine dipstick
- Estimated GFR
- Urea and electrolytes
- Magnesium, calcium and phosphate
- Serum albumin
- Serum creatinine
- Lipid profile (including triglycerides)
- Random blood glucose
- Haemoglobin A1C (if diabetic)

- Bicarbonate
- Uric acid
- Full blood count
- High sensitivity C-reactive protein (CRP)
- Ferritin (if anaemic)
- Folic acid (if anaemic)
- Vitamin B12 (if anaemic)
- Serum parathyroid hormone
- Urine albumin to creatinine ratio

Blood samples are requested for gene analysis (DNA and RNA) and laboratory based biomarker studies (e.g. plasma, serum). Further samples will be collected at follow-up appointments and at the time around transplantation (pre-operatively, within one week post-operatively and six monthly after transplant up to 18 months) and at the onset of each episode of disease relapse and subsequent remission (remission sample should be taken at the next outpatient appointment post relapse visit). Blood samples may also be taken for gene analysis in B and T lymphocytes separated from peripheral blood mononuclear cells (PBMCs). Stored samples may also be requested and used in the study, if deemed more appropriate at the time of collection.

The Bristol lab may also request the collection of urine samples for analysis and collect spare biopsy samples that have been/ will be taken during routine biopsy appointments. This may include access to clinical microscope slides that have been made from biopsy tissue. Any clinical slides will be scanned and stored on a secure server and then returned to the local centre. Labs may also be asked to upload images of immunostaining and electron microscopy performed in clinical diagnosis (if available). For future routine biopsy appointments, consent will be sought to take additional research tissue samples during the procedure. These urine and biopsy samples will be used for further lab analysis, which may include genetic investigations.

Biological samples collected as part of this study may be used to create immortalised cell lines which will help further *in vitro* work.

Plasma exchange samples are requested at each recurrence episode, where samples are taken at first exchange and weekly thereafter for 4 weeks. Patient consent will be obtained in all cases. All samples will be used, stored and disposed of in accordance with the Human Tissue Act.

Sample packs will be provided to centres to encompass sample return. Sites are advised how to return samples through the Standard Operating Procedure (SOP). This will vary depending on whether the centre is a Study Site Type A or Study Site Type B. Detailed information on how to collect the samples, type of blood bottles and the amount required for each visit can be found in the corresponding SOP for each study site type.Study Site Type A: All collected samples will be posted (through appropriate packaging) from each site using Royal Mail or approved courier services, to the University of Bristol and will remain as Bristol University's property. The Chief Investigator, Professor Moin Saleem, will be responsible for ensuring that samples are transferred, stored and maintained in the appropriate conditions. Sample logs will be kept at each site and at the University to ensure traceability of all samples. The Research may include the participation of other academic institutions/commercial companies and sample transfer to these other sites will be documented.

Study Site Type B: Samples will be processed and stored at each participating site according to the sample-handling SOP. Frozen samples (blood and urine) will be transferred on dry ice by courier from each participating site to the NIHR National Biosample Centre in Milton Keynes or the University of Bristol approximately every 4 months. All shipments will be accompanied by a complete inventory of all samples. Samples allocated for NURTuRE collaborators will be sent out from the National Biosample Centre to different laboratories for analysis. Samples that are will be made available to other investigators/commercial companies (biobanked) will be released under Access Committee approval. Only when this has been received in writing will the samples be sent to the requestor. Sample transfer in

both these scenarios will be documented. Any sample left after analysis will either be returned to the National Biosample Centre or destroyed. A master database of all frozen samples will be held at the National Biosample Centre in a password protected file. Surplus kidney biopsy tissue embedded in paraffin blocks as well as stained microscope slides will be shipped at room temperature from each participating centre to the HTA licenced biorepository facility at the University of Birmingham (Human Biomaterials Resource Centre – HBRC). Here the slides will be photographed and digital images will be stored and identified using the patient's RaDaR number. The slides will then be returned by courier to the participating centre. Clinical labs will also be asked to supply digital images of immunostaining and electron microscopy performed in clinical diagnosis, if these are available. Residual renal tissue in paraffin blocks will stored and further sectioned for analyses (which may include genomic analysis) as planned by the study investigators/collaborators and for future access to investigators/academics/commercial companies via the Access Committee. These slides will be shipped to various laboratories for analysis and sample transfer documented. A master database of all biopsy samples will be held at the University of Birmingham Biorepository Centre in a password protected file.

1.4 Phenotype/Genotype analysis

Currently, mutations in over 50 genes are associated with the development of FSGS. These include genes encoding Nephrin, Podocin, CD2AP and PLCe1 in early childhood, WT1 in later childhood (Frasier Syndrome), and TRPC6 in late adolescence (earliest reported case is 17yrs old). DNA will be extracted and sequencing will be carried out to look for mutations in genes that may cause/ be related to Nephrotic Syndrome. Gene analysis of B and T lymphocytes will help in identifying if there are specific genetic rearrangements associated with Nephrotic Syndrome in these cells. RNA may also be extracted to look for gene expression changes. Consent will be sought for participants to agree for their genetic material to be analysed. The University of Bristol Research Team works closely with the Bristol Genetics Laboratory (BGL) at Southmead Hospital, who may extract DNA from the samples. Samples will be transferred at room temperature using the inter-site Hospital delivery service. All samples will be accompanied by a sample identification slip and transfer

of samples between the two sites will be recorded to allow traceability of samples. Samples will be labelled for DNA extraction only and will not be stored at the BGL.

In addition, parents/relatives attending clinic with their child/relative will be asked to consent to donate a blood sample. This sample will be collected by the Research nurses/study staff at the centre. Parents/relatives will be provided with the parent/guardian/family PIS that details the reasons for collecting these samples and will be asked to sign and date the consent form if they wish to be included. Parents/relatives can consent at any stage during the child's participation in the study. It may be that copies of the PIS are distributed by the participant/family to parents/relatives who were not in clinic with the child. Relatives under the age of 16 can participate, with their parent/guardian consenting on their behalf.
The PIS details what is involved and contains contact details of the clinical care team if they would like further information. Blood samples are preferred but if this is not possible, the clinician/clinical team may deem it more appropriate to collect a saliva sample. Self-collection saliva kits will be provided by the Research Group. Genetic material extracted from these samples (or from a stored DNA sample sent to the research lab, as may be the case with relatives also affected by a kidney condition) may be used to check the inheritance of any potentially causative gene mutation discovered in their child/relative's sample and to assist research into the genetic causes of NS. The samples will be sent and stored at the University of Bristol. DNA may be extracted at the University of Bristol or transferred to Bristol Genetics Laboratory (BGL) at Southmead Hospital as outlined for patient samples in section 1.1.2.

Analysis of genotypic and phenotypic features collected by the Registry will take place, which will include: age of onset of disease; detailed treatment timeline; response to treatment; detailed histological categorisation (a national pathology steering group is part of RaDaR); and ongoing laboratory and clinical parameters. Therefore they will utilise these data to identify specific risk factors for disease progression pre-transplant, and recurrence of disease, including genetic correlations.

Using the resources provided by RaDaR, patients can additionally consent to whole genome sequencing using the NIHR BioResource (see below).

1.5 Collaboration with the BioResource for Rare Diseases

The NIHR BioResource – Rare Diseases has been established to identify genetic causes of rare diseases, improve rates of diagnosis and to enable studies to develop and validate treatments; thus improving care for those with rare diseases and their families. In October 2013 our group received funding from the NIHR via their Translational Research Collaboration, which aims for deep phenotyping and genotyping of Rare Disease Cohorts. Using this money, collaboration with BioResource will involve the provision of samples and data collected in our study.

Through the RaDaR database, Bristol researchers will confirm if patients are nationally recruited to the NephroS study. Once confirmation is acquired, postal consent for the BioResource study will be obtained by the Bristol research team. This is to allow the sharing of samples and data collected as part of the NephroS study (formerly SRNS) with BioResource. BioResource patient information and consent forms will be sent which details how patient information will be handled confidentially within the BioResource study.

Sites will not be asked to be involved with BioResource consenting (unless the site has separate approval for the NIHR BioResource – Rare Diseases), but will continue to be asked to process samples collected for research purposes as part of the NephroS study.

Once consented, the Bristol team and Professor Moin Saleem will be responsible to transfer of an aliquot of sample on ice using approved couriers (ensuring traceability and sample quality) to the BioResource centre. A copy of the BioResource consent form will be sent alongside the sample. The original consent form will be kept in the NephroS Study Master File.

The BioResource centre will be responsible for maintaining traceability and sample quality through a sample log and appropriate storage. If patients do not return postal consent for

the BioResource study, samples will not be shared with BioResource and will be stored for use in the NephroS study and ethically approved future National/International studies.

In order to achieve collaboration with the BioResource centre, the Bristol Centre will:

1. Seek retrospective consent from existing participants of the NephroS study (former SRNS) for the release of samples to the BioResource study. Participants will be provided with the BioResource information sheets and consent forms.

2. In future we will seek consent from participants of the NephroS study for the release of samples to the BioResource study. Participants will be provided with the BioResource information sheets and consent forms.

1.6 Biological Analyses

For *in vitro* experiments, the University Research Group will use our previously described human podocyte cell lines, which are widely acknowledged as the most representative cellular model available. Previous work from this laboratory has identified a specific cellular agonist for TRPC6[14], which for the first time allows direct testing of TRPC6 activation in podocytes. Utilising this, they have shown that TRPC6 in cultured podocytes is aberrantly activated by incubation with plasma from children in relapse, post-transplant, compared to activity in response to remission plasma. This difference has been consistent in every patient so far tested (5 patients), and needs to be extended to a larger cohort. This cellular calcium flux assay will be used for TRPC6 activation as a functional test of disease activity in these patients in relapse and remission.

The University Research Group have identified another cellular parameter of nephrotic disease activity in these patients, that of intracellular relocalisation of the slit diaphragm proteins nephrin, podocin and CD2AP in response to relapse plasma. This will also be tested in the larger cohort, using standard methods of immunofluorescence well established in the laboratory.

Finally, activation of a novel cellular pathway has been identified in response to FSGS relapse plasma compared to remission, in podocytes. This involves phosphorylation of VASP, a molecule which links the cytoskeleton to signal transduction pathways. The assay for VASP phosphorylation is now well established in the laboratory, and will be used to test the larger cohort.

Once the pattern of cellular changes in response to relapse plasma is defined, they will move on to test the blood samples from these patients obtained pre-transplant, during episodes of relapse, remission, and plasma exchange fluid during recurrence to determine if the same activity exists.

Thus the group have identified three relatively rapid *ex vivo* tests of patient plasma which will be able to provide a much fuller picture of disease activity patterns than has been previously achievable, with the possibility in the longer term of finding a consistent enough test, or combination of tests, to predict patients who are likely to relapse, before or very shortly after renal transplantation. This would significantly enhance both patient information, and management decisions around the time of transplantation.

In order to identify the circulating plasma factors that are responsible for the recurrence of FSGS in a large proportion of renal transplants, it is necessary to have control samples of discarded plasma from patients who have undergone plasma exchange treatment for conditions other than Nephrotic Syndrome. By comparing these samples in a proteomic screen, the Research Team in Bristol hope to identify specific Nephrotic Syndrome factor(s) that causes recurrence.

Plasma exchange bags, which are normally discarded after treatment, will be collected from patients undergoing routine plasma exchange treatment for conditions other than Nephrotic Syndrome. Anonymised plasma exchange bags will be sent to the University of Bristol, using cool-bags supplied by the Research Team. The researchers at Bristol will not be in possession of, and are not likely to come into possession of, information that identifies

the persons from whom it has come. The patients from whom the control samples are collected will not be under clinical care of any of the researchers. Only the disease type will be provided to enable comparison between patient and control samples. According to the Human Tissue Act, collection of anonymised samples in this way is under consent exemption.

The collection of blood, urine and biopsy samples will help further the biological analysis of Nephrotic Syndrome. Research carried out by collaborators will allow for a wider spectrum of biological analyses on the sample types collected, increasing our knowledge of Nephrotic syndrome and identifying biomarkers that may contribute to Nephrotic Syndrome.

2. STUDY OBJECTIVES

On the basis of the Research Group's biological interest in the mechanism of FSGS, recent advances in the genetics of FSGS, and the urgent need for better phenotyping and genotyping of FSGS on a national basis, the group was awarded a substantial grant from the Medical Research Council to set up a UK Nephrology Rare Disease Registry, with NS/FSGS and MPGN (Mesangio-Proliferative Glomerulonephritis) as the pilot diseases to study. This allowed the first ever national infrastructure for collation of clinical, biochemical, genetic and biological collection of data in NS, closely integrated with our well established laboratory programme of research, in order to address the stated questions. Further funding has been obtained on the basis of the Registry that has been created, to extend the phenotype and age range, as well as perform more detailed genetic studies as described. The latest NURTuRE initiate allows further and more extensive research to take place.

The advantages of undertaking this research will be:

1) To answer the question of whether and to what degree, post-transplant recurrence of FSGS is a heterogeneous disease seen at the level of the cellular response.

2) To provide comprehensive genotype/phenotype correlation of the disease, in a substantial cohort of patients (all adult and paediatric patients diagnosed with idiopathic NS in the United Kingdom).

3) To provide the basis for a predictive *in vitro* test for patients who will suffer recurrence of the disease post-transplantation.

4) To identify a common disease mechanism in this group of patients, which would form the basis for an interventional clinical trial of novel pharmaceutical agents.

3. STUDY DESIGN

3.1 Study outcome measures

This is a prospective multi-centre observational study. All patients that fit the inclusion criteria should be enrolled if willing.

4. PARTICIPANT SELECTION

4.1 Pre-registration evaluations

There are no pre-registration evaluations. As long as patients meet the criteria they can be entered in the study. The Research Team at each centre will identify potential participants through hospital admissions, renal databases, records and RaDaR and approach all children/adult patients that fit the inclusion criteria. The Research Team also have a recruitment poster that can be displayed at the hospital.

4.2 Inclusion criteria

- Children and adults (no age restrictions)
- Idiopathic Nephrotic Syndrome (nephrotic range proteinuria and/or hypoalbuminaemia)

and includes:

- Congenital NS (presumed Steroid resistance)
- Childhood or adult onset with primary Steroid Resistance
- Childhood or adult onset with late onset Steroid Resistance

- Steroid Sensitive Nephrotic Syndrome, early in the disease course i.e. after one episode of Nephrotic Syndrome
- As part of a syndrome e.g. Nail Patella Syndrome and Denys-Drash Syndrome

NB. Those with a biopsy diagnosis of FSGS or minimal change disease can be included if they fall in the above categories but biopsy is not a prerequisite for inclusion.

4.3 Exclusion criteria

Secondary causes of Nephrotic Syndrome e.g. primary diagnosis of Glomerulonephritis (IgA Nephropathy, Membranoproliferative Glomerulonephritis, Membranous Nephropathy), Vasculitis, Systemic Lupus Erythematosus, Diabetes, Obesity, Hypertension.

4.4 Consent

4.4.1 Children consent

Patients, parents/guardians or children will be informed about the study by letter of invitation and will be provided with the relevant patient information sheet. The Research Team may contact the patient to confirm whether they would like to participate and arrange to meet them in clinic to discuss the study further and take consent. Information will be offered in the patient's first language using translation services provided locally within the NHS. Information for children will also be provided. Written informed consent will be obtained either by the Nephrologist or a Research Team member in accordance with Good Clinical Practice research guidelines. All patients must be enrolled in RaDaR either prior to or at the time of consenting. Original consent to be kept in study file, a copy of it will be held in the patient's hospital medical record, and a copy sent to the patient. (If patient consented for RaDaR at time of consenting for NephroS, RaDaR consent form and copies to be filed as for NephroS consent form). The patient's general practitioner will also be informed of their participation through the GP letter and patient information sheet or via the routine clinic letter.

Patients that take part in the NephroS study will be offered the opportunity to opt in to have a comprehensive DNA analysis performed within the NIHR BioResource study. The

invitation to take part in the NIHR BioResource comprehensive DNA analysis study will be given to patients as per **1.5**. Genetic reports will be shared with the patient's Nephrologist, the patients and their parents/guardians.

For children, the period of consent will end when the patient reaches 18 years of age, and consent will need to be given independently by the patient. Children can consent as adults from 16 years of age for both the RaDaR and NephroS studies. If they have not re-consented as an adult by the time they reach 18 years of age their RaDaR registry record will be frozen and their Nephrologist informed. No further samples will be collected for the NephroS study until the patient has re-consented as an adult.

4.4.2 Adult consent

Patients will be informed regarding the study by letter of invitation and will be provided with the relevant patient information sheet. The Research Team may contact the patient to confirm whether they would like to participate and arrange to meet them in clinic to discuss the study further and take consent. Information will be offered in the patient's first language using translation services provided locally within the NHS. Written informed consent will be obtained either by the Nephrologist or a Research Team member in accordance with Good Clinical Practice research guidelines. All patients must be enrolled in RaDaR either prior to or at the time of consenting. Original consent to be kept in study file, a copy of it will be held in the patient's hospital medical record, and a copy sent to the patient. (If patient consented for RaDaR at time of consenting for NephroS, RaDaR consent form and copies to be filed as for NephroS consent form). The patient's general practitioner may also be informed of their participation through the GP letter and patient information sheet or via the hospital clinic letter.

Patients that take part in the NephroS study will be offered the opportunity to opt in to have a comprehensive DNA analysis specifically for renal disease performed within the NIHR BioResource study**.** The invitation to take part in the NIHR BioResource comprehensive DNA analysis study will be given to patients as per **1.5**. Genetic reports will be shared with the patient's Nephrologist and the patient.

4.4.3 Consent for existing NephroS patients at Site-Type B NephroS-NURTuRE centres

Patients who originally consented for the NephroS study are already aware through the PIS and consent form that their samples may be used by other academics and commercial partners and their samples will be stored for future research use. At the point of resampling, verbal continuing consent will be sought at resampling to ensure the participant wishes to continue and will be briefed about the developments within the study.

4.5 Confidentiality

Patient information will be collected under the remit of the RaDaR database. This includes both generic and disease specific information and will include patient identifiers. Access to the RaDaR database is strictly controlled by the RaDaR research team. Only members of the research team requiring this information will have access. Other collaborators/researchers who are interested in Nephrotic Syndrome will have access to anonymised data collected on the RaDaR database.

At the end of the study, the data will remain with RaDaR and any extra data that is generated by the study will be made available to the database. Scientific and clinical data derived from patient samples may be made publically available through open access publishing. However, the patient will not be identified personally in any report, website or publication.

4.6 Withdrawal of patients from the study

Patients may withdraw from the study at any time by notifying the local research team or their lead clinician. As data is held by the registry under separate ethical approval, this will not be affected unless they withdraw from the registry as well. A participant will be withdrawn from the NephroS study whenever there is medical indication or when it is the participant's wish, without prejudice.

If a patient withdraws the results of any tests on samples already collected will be used in analysis. We would prefer to keep any samples in central storage for future use but these could be destroyed if requested, according to the Human Tissue Act and in agreement with local guidelines. Withdrawal should be documented in patient medical notes and centres need to inform the main centre of subject discontinuation, so appropriate action is taken. Any additional studies that the patient signs up to, including BioResource, are under separate approvals and the patient must also notify these studies if they wish to withdraw.

5. STUDY SCHEDULE AND DURATION

5.1 Study schedule

Samples and data will be collected from consented patients according to study flow chart shown in Figure 1 and study activity table in figure 2.

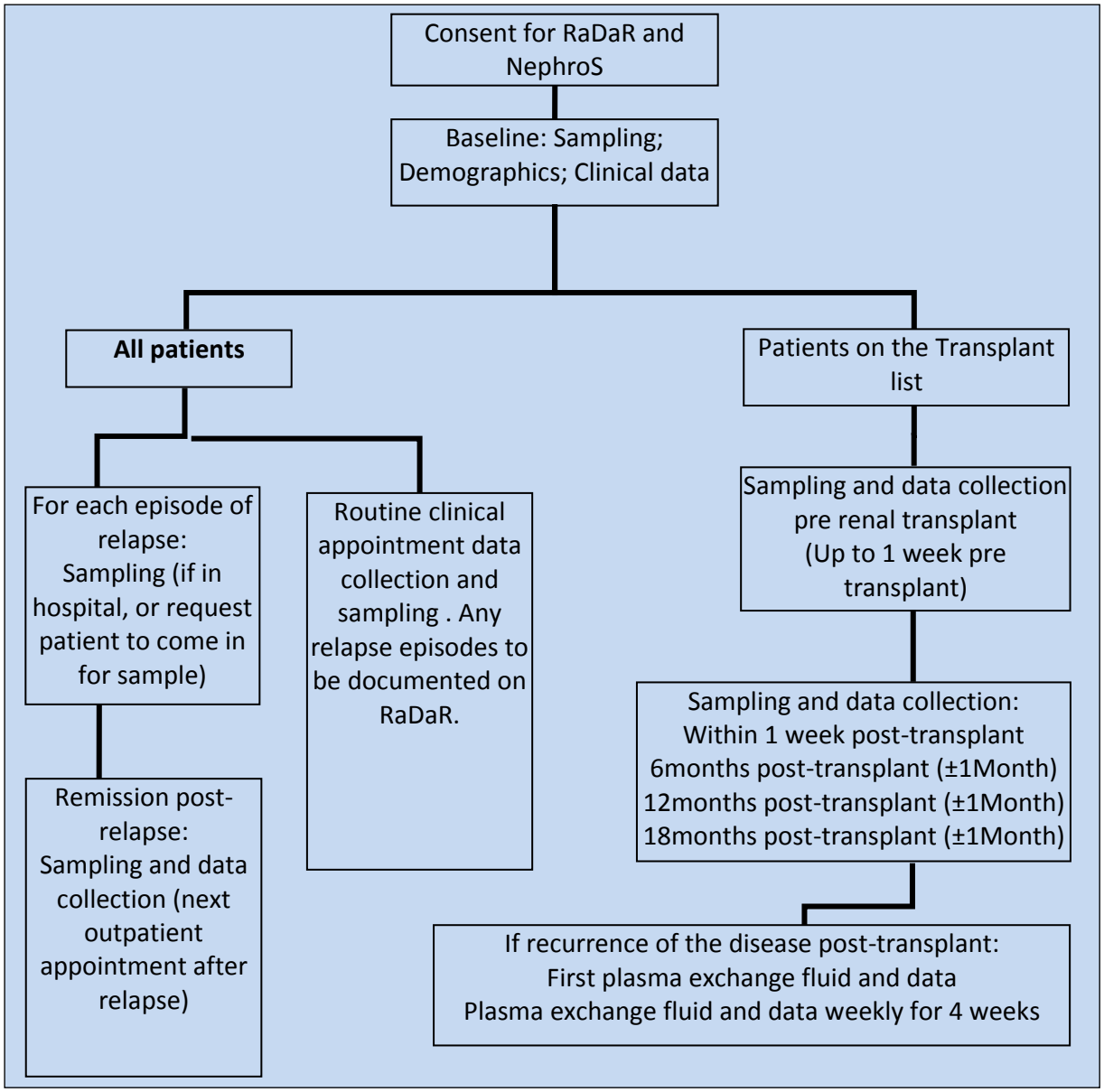


Figure1: NephroS Study flow chart

Activity	Baseline	Relapse	Remission post relapse	Routine clinical appointment update (approx. every 6 months or when seen in clinic)	Pre-transplant	Post-transplant (1 week and 6/12/18 months after)	Post-transplant recurrence (first exchange and weekly thereafter for 4 weeks)	Biopsy
Screen	x							
Consent	x							
Blood samples	x	x (in clinic, or request patient to attend appointment to give a sample)	x	x	x	x	x	
Urine	x	x (in clinic, or request patient to attend appointment to give a sample)	x	x	x	x	x	
Tissue Sample								x
Plasma exchange fluid							x	
Demographics	x							
Update demographics		x	x	x	x	x	x	x
Clinical data	x	x	x	x	x	x	x	x (pathology report)

Figure 2: Study activity table outlining when samples may be taken. Full details provided in full Sample Handling SOP, including a schedule, sample volumes and delivery instructions.

5.2 Sample/Data collection, processing and storing

Sample collection, will be performed as per local blood collection guidelines. The samples should be labelled and stored/sent as per sample collection SOP and outlined below (section 5.5).

The samples will be stored in a secure laboratory and their location will be logged and recorded on a secure database.

5.3 Quality of Life

Adult patients will be asked to complete a socio-demographics (including age, gender, ethnicity, first language, education status, marital status, employment, indices of multiple deprivation (IMD) score, smoking history, alcohol intake, and dietary status) and validated quality of life questionnaires, current symptoms, anxiety and depression, cognitive function and health literacy at key-time points throughout their disease course.
For children, parent/guardian or child completed questionnaires will be supplied appropriate to the age of the child for quality of life and health utility. These will be given at key-time points throughout their disease course. For both adults, children and their parent/guardian, assistance will be provided if needed. These questionnaires will allow an assessment of the relationship between the severity of Nephrotic Syndrome, co-morbidities and quality of Life. The results of the questionnaire will be inputted onto the RaDaR database by a member of the local research team. These questionnaires may be completed in clinic or at home via post/phone/fax/email as appropriate. Questionnaires may be posted to patients (including ahead of the initial visit), and the research team may follow-up with patients regarding their completion.

5.4 Duration and Follow-up

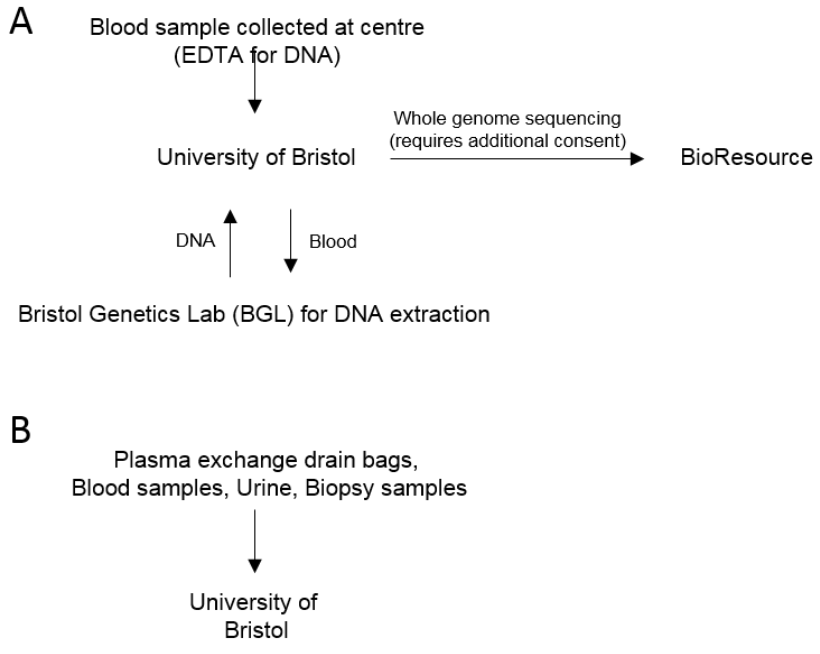
All patients enrolled in the study will have a baseline visit and further sites visits as appropriate depending on disease course. Clinical data, demographics and samples will be collected. Data collected should be entered in the RaDaR database and repeated with samples collection according to the study flow chart and sample handling SOP. Enrolment

into the NURTuRE part of the study will begin from January 2017 and recruitment will continue for 3 years until 31 December 2019 or until at least 800 patients have been recruited into the NURTuRE side of the project (i.e. at least 800 patient samples biobanked). Recruitment for study site A will continue through this time, with the aim to capture all prevalent and incident cases of Nephrotic Syndrome according to the inclusion/exclusion criteria. Patients will continue to be followed up for clinical information via their participation in the RaDaR registry.

5.5 Sample Transfer within the NephroS Study

Figure 3 highlights the samples transfer procedures for Study Site Type A and Site Type B.

<u>Site Type A</u>



Site Type B (NURTuRE)

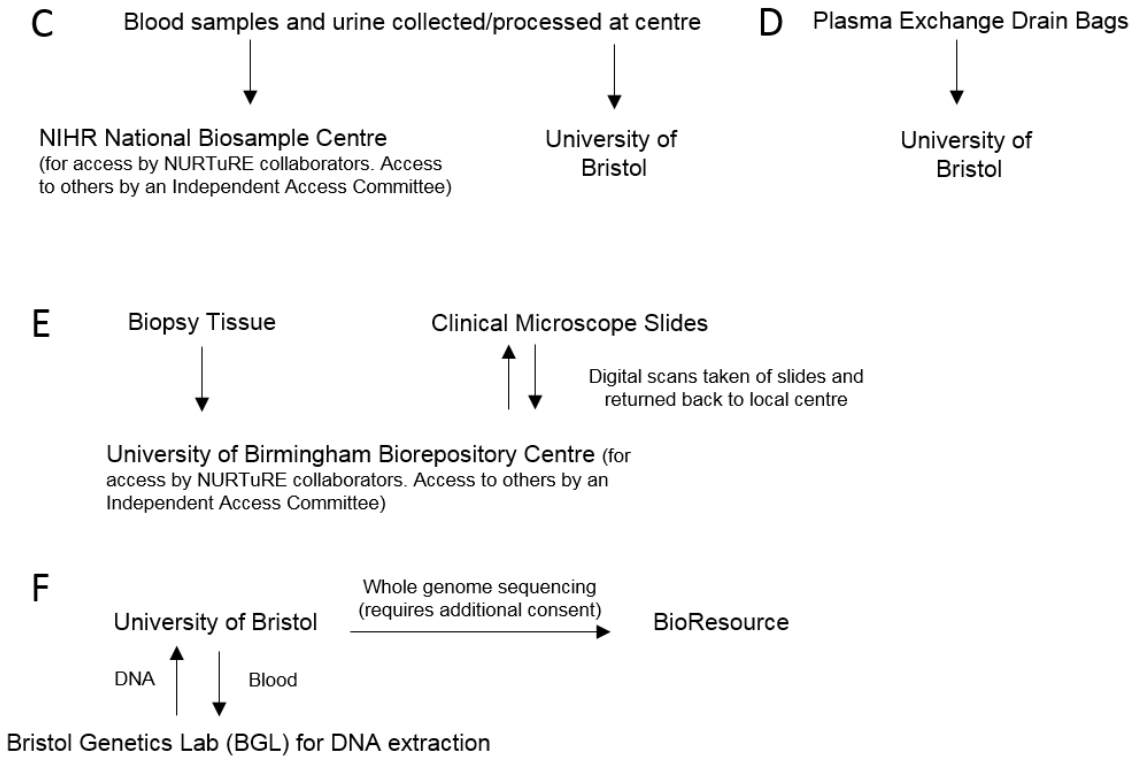


Figure 3: Flow diagram of sample transfer within the NephroS study Site Type A (top panel) and Study Type B (bottom panel). Sample packs, including postage/courier costs will be provided by the research team. How to transfer samples in each case will be detailed in a sample handing SOP. Study Site Type A, samples will be labelled with RaDaR number and Date of Birth as a second identifier (as previously approved). Study Type B samples will be barcoded with a link to RaDaR number and year of birth.

6. ADVERSE EVENTS

6.1 Definitions

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*

- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious. As this is an observational study, the only AEs with a causal relationship to study procedures i.e. those directly related to study visits, which are expected to be extremely uncommon.Only SAEs related to study visit research procedures will be reported.

6.2 Reporting procedures

The majority of blood samples will be collected during routine blood collection, whenever possible. However, if this is not feasible they may be asked to visit the hospital on another occasion. It is not expected that patients will suffer from one of the serious or non-serious adverse effects. If a patient gives a blood sample aside from routine bloods, these will be carried out by an experienced member of staff and have the same risks as associated as those taken for routine blood samples. Any extra tissue biopsy samples will only be taken during routine biopsy procedures.

7. STATISTICS AND DATA ANALYSIS

Simple statistical analysis of paired samples will be performed to compare certain clinical entry criteria with outcome (disease progression, re-occurrence post-transplantation). Similarly outcome will be compared with treatment received to identify possible trends. Genotype/Phenotype correlation (comparing outcome for children with each gene mutation) will be performed. This is expected to be performed by the University staff using simple statistical analysis.

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

8. REGULATORY ISSUES

8.1 Ethics approval

This study has been approved by the South West- Central Bristol Research Ethics Committee, reference number 09/H0106/80. The study must be submitted for R&D approval at each participating NHS Trust. The Chief Investigator/ Principal Investigators will require a copy of the Trust R&D approval letter before accepting participants into the study.

The genetics BioResource has been reviewed and approved by Cambridgeshire 2 Research Ethics Committee. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

8.2 Indemnity

The University of Bristol has arranged Public Liability insurance to cover the legal liability of the University as Research Sponsor in the eventuality of harm to a research participant arising from management of the research by the University. The University of Bristol's Public Liability insurance policy provides an indemnity to our employees for their potential liability for harm to participants during the conduct of the research. This does not in any way affect an NHS Trust's responsibility for any clinical negligence on the part of its staff (including the Trust's responsibility for University of Bristol employees acting in connection with their NHS honorary appointments).

8.3 Sponsor

Bristol University is the Research Sponsor for this trial.

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Delegated responsibilities will be assigned to the NHS trusts taking part in this trial.

8.4 Funding

NIHR and a grant by Kidney Research UK supported by funding from several pharmaceutical companies who are active collaborators in the study including UCB Pharma Ltd, Evotec International GmbH, AbbVie.

8.5 Audits and inspections

The study may be subject to inspection and audit of regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition). Data monitoring will be performed by the lead centre research team. The University of Bristol (UoB) has a Service Level Agreement in place with a local NHS Trust (UH Bristol). As part of this, UH Bristol will undertake monitoring of research projects were UoB is fulfilling the responsibilities of a Research Sponsor. A minimum of 10% of UoB Projects will be monitored.

9. STUDY MANAGEMENT

The clinical data will be held by the RaDaR (Rare Renal Diseases Registry) database. This database has secured full ethical approval for data collection and for patients to be approached for studies. Only members of the Research Team directly involved in the study will be able to access the data through their website for the period of the study. Access to the database is strictly controlled by the RaDaR research team. For analysis, the RaDaR team are able to extract information from the database to make the data available to the Research Group, collaborators and access to future academics/commercial companies in collaboration with the RaDaR group.

RaDaR is a development of the Renal Association, and is operated by the UK Renal Registry (UKRR). Governance of RaDaR will be undertaken under the authority of the Renal

Association of Great Britain, the professional body for Nephrologists in the UK, via its Clinical Affairs Board.

The business aspects and strategic direction of UKRR are overseen by the UKRR Management Board, comprising the Trustees of the Renal Association together with the Director and General Manager of the UKRR. The Management Board is chaired by the immediate past President of the Renal Association. The UKRR Management Board meets face to face annually. Additional virtual meetings of the Management Board are held as deemed necessary by the Chair throughout the year by phone conference or email.

The RaDaR Committee will be a subcommittee of UKRR Committee, and will be responsible for all operational aspects of the rare disease registry. The committee will consist of a Chairperson, an honorary secretary and one other member all of whom are members of the Renal Association and appointed by it. In addition the RaDaR Committee will have representatives from participating Disease Specific Research Groups (which this Research Group is) or their deputies, two user representatives (patients or carers), a doctor training in nephrology or paediatric nephrology, and a renal health professional with no medical qualification. The RaDaR Committee will report to both the UKRR Committee and to the Research Committee of the Renal Association.

9.1 Changes to study protocol

Amendments to, or formal clarification of, the study protocol will be documented in writing. University of Bristol will remain responsible for all amendments or administrative changes to the protocol, and will distribute up-to-date documentation upon appropriate competent authority/ethics committee approval. The amendment will be sent to participating NHS organisations so that, where necessary, arrangements can be put in place to continue the site's capacity and capability to deliver the study. All investigators will need to acknowledge the receipt of new study documentation, and remain responsible for gaining local Trust approval before implementing any changes. Written confirmation of local trust approval must be sent to the sponsor- University of Bristol.

10. PUBLICATION POLICY

Data from the NephroS study will be the property of the NS Rare Disease Group (for up to date membership details see www.rarerenal.org), and will be analysed and published in an open manner. Authorship will explicitly state the publication is 'on behalf of RaDaR and the NephroS study group', and individual authorships for local leads in the study will be sought wherever possible.

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