

# A National DNA Bank for Glomerulonephritis

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## Disease/Condition and the Case for Genetic Research

### *The disease targets*

We will collect DNA from patients with the most common forms of glomerulonephritis (GN) whose phenotype can be precisely defined. These are: IgA nephropathy, membranous GN, minimal change disease, focal necrotising GN, and systemic lupus erythematosus (SLE) with secondary GN. These conditions have clinical features in common and there is compelling evidence that they also share pathophysiological mechanisms. They all cause proteinuria and all except minimal change disease can progress to end stage renal failure. Thus, the collection of DNA from patients with these disorders and their relatives will permit future identification of genetic determinants uniquely associated with particular diseases as well as genetic determinants that influence progression to renal failure.

### *Diagnostic criteria*

Diagnosis of the different types of glomerulonephritis will be based on the morphological and immunohistological appearances on renal biopsy, together with the clinical and serological aspects of the disease. To ensure an accurate disease phenotype, renal biopsy assessment using WHO criteria will be mandatory for the diagnosis of IgA nephropathy, membranous GN, focal necrotising GN, and minimal change disease in adults. However, although desirable, a renal biopsy will not be mandatory for diagnosing lupus nephritis or for diagnosing minimal change disease that presented in childhood. Selection criteria in the individual diagnostic groups will be as follows:

- (a) IgA nephropathy: patients will be aged between 18 and 50 years with biopsy evidence of mesangial proliferation with IgA deposition; cases associated with Henoch-Schönlein purpura will be included, cases secondary to liver disease will be excluded.
- (b) Membranous GN: patients will be aged between 18 and 70 years with renal biopsy evidence of basement membrane thickening with immune complex deposition, patients with disease secondary to known causes (such as tumours) with the exception of SLE will be excluded.
- (c) Focal necrotising GN: patients will be aged between 18 and 70 years with histological evidence of necrotising GN with epithelial crescent formation and the presence of circulating anti-neutrophil cytoplasmic autoantibodies (ANCA).
- (d) Minimal change disease: Adults between ages of 18 and 50 will be selected with proteinuria or the nephrotic syndrome in whom renal biopsy has excluded alternative diagnosis (specifically focal and segmental glomerulosclerosis (FSGS) and where the light microscopy is normal consistent with the diagnosis of minimal change disease; alternatively patients over 18 years of age who had presented originally with idiopathic steroid responsive nephrotic syndrome before the age of seven years even if they have not undergone a renal biopsy.
- (e) Lupus nephritis: patients will be between the ages of 18 and 50 and have a definite diagnosis of systemic lupus based on the clinical and serological features of lupus nephritis defined by the American Rheumatism Association (ARA) criteria (Tan *et al*, 1982). Most patients will have renal biopsies, which will be classified according to the WHO criteria.

### *Importance as a public health issue*

The treatment of end stage renal failure (ESRF) poses substantial problems not only for patients and their families, but also for the health care system because it will ultimately consume more than 5% of the total NHS budget. Glomerulonephritis (GN) is numerically one of the largest causes of ESRF (Disney, 1999), especially amongst the young and middle aged: current treatments are relatively ineffective. Thus, lupus nephritis is most frequent in young women in their 20s and 30s, IgA nephropathy affects patients of all ages, and membranous GN and focal necrotising GN are prevalent in the fifth and sixth decades of life. The onset of renal failure as a result of these diseases has a major impact on quality of life and creates a long-term dependence on renal support and is associated with excess mortality especially from accelerated cardiovascular disease.

Minimal change disease affects both adults and children and does not cause chronic renal failure. It is the commonest cause of acquired childhood nephrotic syndrome and responds to steroid therapy, which will make it a useful comparator to progressive forms of GN. Nevertheless, minimal change is associated with significant childhood morbidity as a consequence of severe proteinuria and the therapeutic use of high-dose corticosteroids. Despite differences in the natural history of the types of GN described, it is likely that some common aetiological factors operate across the spectrum of these glomerular diseases. Understanding the nature of this predisposition should enable the development of more effective treatments. The overall purpose of this application is to facilitate this by establishing a UK national DNA collection for the most common forms of glomerulonephritis.

### *Evidence for genetic aetiology*

Evidence for genetic predisposition to kidney disease is derived from many sources, including studies of familial incidence and racial predisposition in man, and differing susceptibility in inbred animal models. The extent of genetic predisposition is generally estimated from the risk ratio,  $\lambda_s$  (risk of disease in a sibling of a patient / risk in the general population). A recent survey of patients with ESRD (excluding established monogenic diseases such as polycystic disease and Alport's syndrome) found that 351 out of 4289 patients reported a sibling with ESRD (Freedman *et al*, 1997). Assuming an average sibship of 2.5 this gives a risk to sibling of approximately 3% against a population prevalence of ESRD of approximately 0.2% ( $\lambda_s = 15$ ). There is evidence to support a genetic predisposition with respect to the individual glomerulonephritides.

In IgA nephropathy there are reports of strong familial clustering particularly in inbred communities (Julian *et al*, 1985; O'Connell *et al*, 1987; Scolari *et al*, 1992). Population surveys have identified a high incidence of glomerulonephritis in relatives of cases of IgA nephropathy (Levy, 1993; Rambašek *et al*, 1993). For example, in one survey 9.6% of patients with IgA nephropathy had at least one sibling with GN (Rambašek *et al*, 1993). In some studies renal biopsy material not available in relatives, however, in those surveys that documented renal histology in family members, histological pattern was found to be conserved within families (Julian *et al*, 1985; Scolari *et al*, 1992). Of equal importance, the presence or absence of microscopic haematuria accurately predicts whether or not the disease is present. In summary, the sibling risk appears greatly in excess of the reported prevalence of clinically apparent IgA nephropathy of 25-50 cases per 100,000 population (Glasscock *et al*, 1996). Even allowing for ascertainment bias and diagnostic uncertainty, the epidemiology data suggests a high value of  $\lambda_s$  ( $= 10$ ) in IgA disease.

In SLE, where accurate diagnosis does not necessarily require biopsy, several studies on familial clustering have been conducted in Europe and the USA. The conclusion from these data is that the  $\lambda_s$  in SLE is between 15 and 20 (Vyse and Todd, 1996). Other data implicating a genetic contribution to susceptibility in SLE include the greater prevalence of SLE in Afro-Caribbean,

Oriental Asian and Asian populations from the subcontinent (Hopkinson et al, 1994) compared to white Caucasians in the UK. Indeed the genetic contribution to SLE has recently been further demonstrated by the publication of three genome-wide screens in affected sib-pair and multi-case families (Gaffney et al, 1998, Moser et al, 1998, Shai et al, 1999). When these studies are compared, it is apparent that 13 genetic loci have been corroborated as linked with SLE (Gaffney et al, 2000), although these probands did not all exhibit nephritis. The size of the regions mapped in these studies is too large to permit immediate gene identification, however, they do provide excellent candidate regions for fine mapping (see below). Finally, there are several well-characterised murine models of SLE with features very similar to the human disease (Vyse and Kotzin, 1998). These models exhibit a genetically determined spontaneous autoimmune phenotype.

Two large family studies of childhood nephrotic syndrome reported sibling recurrence risk of 3.3 and 6%, respectively (White 1973; Bader et al 1974). The reported population prevalence for these diseases is again very much lower, suggesting a  $\lambda_s$  value of between 20 and 40 for childhood nephrotic syndrome. These figures may have been inflated by the inclusion of cases of congenital nephrotic syndrome, but the majority of familial cases conformed clinically and histologically to the usual spectrum of cases of idiopathic nephrotic syndrome. Minimal change disease is by far the commonest cause of acquired nephrotic syndrome in childhood. Evidence that membranous GN and ANCA-associated necrotising GN are familial is derived largely from case and MHC association studies (Scolari et al, 1998; reviewed by Phelps & Rees, 2000). Membranous GN has consistently shown strong associations with HLA class II alleles in all populations studied, and relatively small case-control studies have also suggested associations with immunoglobulin, complement, and T cell receptor genes. Inheritance of particular  $\alpha_1$ -antitrypsin alleles has consistently been associated with focal necrotising ANCA positive GN, as have HLA class II and C3 allotypes with lesser certainty. The strong familial predisposition to ESRF overall would further support genetic factors operating in membranous GN and in ANCA-related GN.

We are aware that  $\lambda_s$  values may also be inflated by shared environment influences, leading us to be very conservative in the power calculations for this study (see below). However, our overall assessment of the published literature suggests that the  $\lambda_s$  value is in excess of 10 for several common renal diseases, or for renal disease in general. Increased risk appears well sustained beyond first degree relatives (Scolari et al, 1992; Levy, 1993; Freedman et al, 1997) suggesting that confounding environmental effects may be relatively small and that effects from individual genes might be relatively large (Risch, 1990; Risch & Merikangus, 1993).

#### *Nature of research that would ensue from this resource*

The choice of five common glomerular renal diseases with different natural histories provides the opportunity to study separate conditions but also to identify unique and common genetic determinants of susceptibility and progression. It is likely that different genetic loci contribute to disease susceptibility and to the rate of progressive loss of renal function thereafter ('progression'). Moreover, the loci that cause disease progression may be common to more than one disease and also to other complex diseases, for example hypertension, known to be associated with more rapid progression of chronic renal disease. The creation of a DNA bank will facilitate the identification of genetic loci linked with GN susceptibility and with disease progression. The collection of genetic material from nuclear families will be ideal for fine mapping regions identified in linkage studies. It will also permit the study of candidate genes identified in pathophysiological studies, genetic animal models, and in ongoing studies of related complex genetic diseases (such as essential hypertension (e.g. the MRC Bright study) and diabetes (Davies et al, 1994; Cordell & Todd, 1995; Julian et al, 1991).

## **The Need for the Collection**

There is no existing UK collection of genetic material from families with the glomerulonephritides. The genetic contribution to susceptibility and progression to end-stage renal failure establish the need for such a collection to facilitate genetic analyses. There is an existing collection of genetic material from SLE families in the UK (PI Dr Tim Vyse). This collection is not focused on lupus nephritis, which occurs in about one-third of lupus patients. Hence a collection of lupus nephritis family material would provide interesting contrasts with those without serious kidney disease. For example, one could address the question: what genetic factors operate to target the kidney in SLE.

The literature on the genetic basis of glomerular disease is mostly based on case-control studies. Some of these have explored the role of candidate genes; for example TGF-beta and components of the renin-angiotensin system (Keavney et al, 1998; Rigat et al, 1990; Tiret et al, 1992; Schmidt & Ritz, 1997; Jardine et al, 1998) using case-control association studies. The available studies have been inadequately powered and have often studied polymorphisms where there are no studies of intermediate phenotype to support the study of individual polymorphisms and disease. As a result, these reports have yielded inconsistent associations between candidate loci and either the presence or progression of renal disease (Jardine et al, 1998). However, such studies have confirmed the potential of this approach in renal disease and the importance of this type of analysis to confirm the future findings from family based studies.

## **Associated Data and Databases**

### *Data storage*

The data obtained from families will related to family structure and clinical details. Clinical information will provide details of the diagnosis (renal histology), the duration, degree and rate of progression of renal failure and laboratory indicators of severity, serum creatinine and albumin levels, as well as haematuria, 24 hour urinary protein loss and glomerular filtration rate. Serial information on serum creatinine, and proteinuria will be extracted retrospectively from the hospital records. Serologic information will also be extracted to provide details for disease classification, including antinuclear autoantibody levels and ANCA. The need for renal replacement treatment as well as current drug medication and past medical history will all be collected. Updates on blood and urine biochemical indicators of renal function and blood pressure will be obtained, and patients will be followed to determine those who die and develop ESRF. Patient date and data on family members will be coded, recorded and stored on an Access database designed for the study.

Parents and siblings (when only on parent is available) will be asked to complete a questionnaire regarding renal disease, provide a blood sample for plasma and DNA, and patients with membranous nephropathy and focal necrotizing glomerulonephritis will be asked to provide the name of a matched control who will also be asked to fill in the questionnaire. Ethnicity (defined by grandparental origin) will be recorded for all cases; the collection will not be targeted towards particular racial groups at this stage.

### *Confidentiality*

All clinical and family information will be stored on non-networked computers in password-protected databases. The database will include individuals' names. However, all participants will be allocated a number code that will be given to the actual biological samples. Genetic analyses will be conducted using these anonymised sample codes. Genetic studies on these samples will only be performed on aliquots of samples and investigators will only be given the necessary clinical information on an anonymous basis. At present there are no plans to pool research data into a

database. The value of pooling and comparing data is extremely important. This collection will be overseen by a steering committee (see below,) which will facilitate the relevant comparison analyses in order to extract as much use from the collection as possible. Ethical Committee approval has been given by the Oxford MREC, and the study will only be performed in institutions whose LRECS have local approval.

## **Practical Details**

### *Participants in samples collection*

The project will be managed by a Steering Committee consisting of all nine applicants under the chairmanship of Professor. Andrew Rees (University of Aberdeen). Each of the five DNA collections will be managed by one of the applicants (IgA nephropathy, Professor Peter Ratcliffe, University of Oxford; Membranous nephropathy, Professor Peter Mathieson, University of Bristol; Minimal change disease, Professor Steven Powis, University College London; Lupus nephritis, Dr Timothy Vyse, Imperial College London; Focal necrotising glomerulonephritis, Professor Charles Pusey, Imperial College London). The collections will be held in the co-ordinators unit and managed by a technician who will receive blood and prepare and store lymphocytes. The technician at the Imperial College will manage both SLE and focal necrotising glomerulonephritis collections.

Patients will be recruited from a network of renal units throughout the United Kingdom that have been divided to cover four geographical regions. Each region will be managed by one of the applicants and patient recruitment will be co-ordinated by a research nurse responsible to him. The four geographical regions are: London (managed by Professor Powis); Oxford, Bristol and Wales (managed by Professor Ratcliffe); Leicester, Birmingham and Manchester (managed by Professor Feehally); and Scotland and North East England (managed by Dr Jardine). Initially patients will be identified within the applicant's own unit and subsequently in other large renal units in their geographical area with the support of our named collaborators (Birmingham, Dr D Adu; Cardiff, Dr K Baboolal; Manchester, Dr D O'Donoghue and Newcastle, Dr T Goodship). Dr P Furness (Senior Lecturer in Pathology, University of Leicester) who operates the UK renal pathology NEQUAS scheme will advise on pathological issues. The network, which covers a total population of in excess of 20 million.

### *Estimate a sample size*

We propose to create a resource that may be used, primarily, in family-based approaches (Risch & Gang, 1995; Teng & Risch, 1999), but retain the option to compare with an unrelated population. This will be based on the collection of nuclear families, consisting of the proband and both parents if they are available, or alternatively of one parent and unaffected siblings if one parent has died. The choice of a family-based approach is based on the available literature from population based association studies of candidate genes in renal disorders, that have been misleading, contradictory and, largely, uninformative. Thus, the planned collaboration will allow large studies with the potential to generate high levels of statistical, and the use of family based methods will obviate the risk of false positive associations arising from unseen populations stratification.

The collection of DNA from probands' parents will allow the use of the transmission disequilibrium test (TDT) to examine allelic association in the presence of linkage (Spielman et al., 1993). Though more laborious than case/control studies, this approach provides a high degree of security against false positive results and is widely regarded as the method of choice for genetic association studies. This approach should be feasible for IgA nephropathy, minimal change disease and lupus nephritis

whose parents are likely to be alive and available for the study. It may not be possible for patients with membranous and focal necrotising glomerulonephritis, even using unaffected siblings in a sibling-TDT. As a back up we will also collect DNA from unrelated controls that could be used in conventional genetic association studies for these two diseases.

As described, the available evidence supports a  $\lambda_s > 10$ , using this value and a genotype specific risk of 1.5 – 4.0 (expected in complex traits) we have estimated that 500 families should provide an 80 – 90% power to establish linkage/association (Teng & Risch, 1999). This estimate is subject to the expected limitations of modelling disequilibrium mapping in complex traits. For each disease we aim to collect a minimum of 500 families, with three or more members over a three-year period. Although the power estimate is based on trios consisting of both parents and patient, comparable numbers will be required for both sib-pair studies and outbred association studies.

The involvement of these large renal units should provide sufficient patients because pilot experience in Oxford suggests that a centre (serving a population of approximately one million) can collect around 100 patients with IgA nephropathy, and both parents over one year. The precise number of patients is difficult to establish on a national basis, and will vary according to the ethnic mix for some conditions (e.g. SLE). However, as an example the Western Infirmary, Glasgow serves a population of 0.85 million for nephrology (and 2.8 million for renal transplantation). This Unit has fully computerised patient records and the numbers of eligible patients with each condition are IgA GN (190), membranous GN (165), minimal change disease (110), focal necrotising GN (65), and SLE (55). It is estimated that the collaborators on the proposal already have access to a population of 15-20 million in the UK. This will provide access to between 1,000 and 4,000 families with each type of glomerular disease targeted. Assuming a recruitment fraction of 30% as a minimum, the figures indicate that a goal of 500 families per disease should be achievable.

#### *Patient recruitment*

Patients will be identified through the renal biopsy registers as the participating centres. This method will favour accurate diagnosis and will permit efficient proband identification. To facilitate use of the histological records we have enlisted the help of Dr Peter Furness, Senior Lecturer in Renal Pathology, Leicester. Dedicated study nurses will be responsible for contacting patients; collection of samples and clinical data from affected patients, as well as the identification of family members and contacting the family members and controls. Initial contact will be by letter and thereafter by telephone. DNA collection will be performed by post, the samples being taken either by participating nephrologists or by the general practice of the controls and family members. In some cases, it will be appropriate for study nurses to collect samples directly from patients and relatives. This model has worked well for IgA nephropathy in the Oxford pilot study. Overall four family/patient-control groups will be recruited each working day over a three year study, thus of the four FTE study nurses, each will require to complete five family/patient-control groups per week. We propose one study nurse to target an individual region. We have defined four geographical area that will be targeted: London, Oxford-Bristol-Cardiff, Leicester-Birmingham-Manchester, and Newcastle-Scotland. Each nurse will be responsible for co-ordinating date collection and recruitment of all patients and communicate with technicians and co-ordinators in the designated collection centres.

### *DNA Samples*

Each adult participant will be requested to donate a 35ml blood sample in two parts. (a) 25ml anticoagulated sample for DNA extraction from neutrophils ( $\sim 400\mu\text{g}$  DNA) and  $\sim 20 \times 10^6$  lymphocytes; and (b) 10mls for serum. Lymphocytes will be separated and cryopreserved in nitrogen to permit future immortalisation if additional DNA is required. DNA will be stored in the aqueous phase at  $-20^\circ\text{C}$  in a refrigerated racking system. Sera will be stored at  $-80^\circ\text{C}$ . These samples will initially be stored in the allocated collection centres.

### *Control samples*

Samples from unrelated controls will be useful adjunct to the family DNA collection but will be restricted to the patient groups with membranous nephropathy and focal necrotising glomerulonephritis. We will recruit a similar number of controls as probands in patients with these diseases. They will be recruited by asking participating families to identify healthy individuals of the same racial background who would be willing to fill in a questionnaire and donate a blood sample.

## Justification

There is a great need for better understanding of the pathogenesis of glomerulonephritis and for the development of better treatments, as these disorders remain a leading cause of end stage renal failure. Evidence that siblings of patients with proven glomerular disease are at greater risk of developing the same condition which provides compelling evidence for the role of inheritance in susceptibility and justification for the approach. The reason for studying five different forms of glomerulonephritis is to maximise the chances of distinguishing genes that confer susceptibility to individual diseases from those that influence whether the disease progresses to end stage renal failure. The five diseases have been chosen because they are relatively common and can be defined with precision.

The applicants are experts in the diagnosis and management of the forms of glomerulonephritis chosen for the study. Equally importantly they collectively have considerable experience, and the skills necessary, for the conduct of large-scale genetic studies. Dr Farell has considerable expertise in statistical analysis of large genetic studies. The medical staff will operate on a voluntary basis but staff will need to be appointed for two purposes:

- Four research nurses will be required to contact patients and their relatives and for the collection and organisation of blood samples and their distribution. Their tasks dictates that they will need to be F grade nurses and they will be based geographically throughout the UK. They will be responsible for identifying and arranging blood sample collection from patients with all disease types; for collecting clinical data and entering it into the database. They will be based in London (University College London -Royal Free Campus), Oxford, Leicester and Glasgow.
- Four full time technicians (E grade) will be required to prepare DNA, to purify and store lymphocytes and to maintain the database of samples. The technicians will receive blood from patients with a single disease except in the case of lupus nephritis and focal necrotising GN (which will be covered by a single technician based at the Hammersmith Hospital London). The remaining technicians will be based in Oxford (IgA nephropathy), Bristol (membranous GN) and the Royal Free Hospital, London (minimal change disease).

Storage of DNA, plasma and lymphocytes will require dedicated freezer and cryo-storage at each collection centre during the project and will be assembled at a single site at the conclusion, as a national resource. It is assumed that centres will have existing I.T facilities appropriate for data storage and electronic transfer. However, substantial periods of computer time will be required for data entry at all centres.

### *Time frame*

Time	Priority	Review 6 monthly	Ongoing work
0-6 months	Establish size of individual local populations Establish family structure Allocate resources	Numbers & recruitment rate Requirement for additional centres	DNA extraction Lymphocyte purification Storage
6-30 months	Collect data and DNA samples from readily available patients & families	Numbers & recruitment rate Requirement for additional centres	DNA extraction Lymphocyte purification Repeat samples (if needed)
30-36 months	Target missing family members, target disease groups with low numbers	As above	As above

### **Management of Collection**

Once complete the five separate collections will be amalgamated into a single glomerular collection which will be divided into two. One collection will remain under the guardianship of the applicants and the other will be located elsewhere, stored under the conditions and regulations agreed between the MRC and the NKRF within the framework governing the MRC DNA collections in order to create a resource for the British Scientific Community.

Information about the DNA bank and how to apply to use it will be freely available on the NKRF and Renal Association web sites.

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### Recruitment criteria

	Age at recruitment	Available relatives	Unrelated Control	Biopsy: within 10 years of recruitment (1990 onwards)	Other inclusions	Exclusions
IgA nephropathy	18 – 50	<b>Essential:</b> both parents  May need to consider one parent + 2 sibs later	<b>Yes -</b> if available	<b>Essential</b> Diffuse deposition of mesangial IgA	Can include HSP - evidence of vasculitic rash or arthropathy	Liver disease
Membranous nephropathy	> 18	<b>Essential:</b> none	<b>Yes -</b> if available	<b>Essential</b> Basement membrane thickening with immune complex deposition		Secondary to other causes: Malignancy Rx: gold, SAID, penicillamine, Hepatitis B, SLE, hypothyroidism
Vasculitis	> 18	<b>Essential:</b> none	<b>Yes -</b> if available	<b>Essential</b> Histological evidence of necrotising GN with or without epithelial crescent formation and no or few immune deposits		
Minimal change disease	18-70	<b>Essential:</b> none  If available, one of following: a) both parents b) one parent + 2 sibs c) 3 sibs	<b>Yes -</b> if available	<b>If available:</b> Compatible with clinical diagnosis. Exclusion of focal and segmental glomerulosclerosis (FSGS)	Steroid responsive nephrotic syndrome compatible with clinical diagnosis and normal renal function, before 7 yrs if no biopsy	Abnormal renal function when in remission
Lupus nephritis	18-70	<b>Essential:</b> none  If available one of following: a) both parents b) one parent + 2 sibs c) 3 sibs	<b>Yes -</b> if available	<b>If available</b>	Clinical and serological features as defined by ARA criteria	

Table 1