Supplemental Material

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Detailed Methods

Patient Cohort

The RaDaR cohort of patients with nephrotic syndrome was used as the source of cases. At January 2018, a total of 2457 patients had been recruited. Patients included for analysis had steroid resistant nephrotic syndrome and an age of onset less than 18 years. The initial cohort, as described previously by Bierzynska *et al*,(1) consisted of 187 patients who had had genetic analysis by whole exome sequencing as part of the NephroS study. Three patients from this cohort were excluded as they had steroid sensitive nephrotic syndrome with focal segmental glomerulosclerosis (FSGS). We expanded this cohort to include the additional patients who have had whole exome or whole genome sequencing at Bristol Renal since this time (four whole exome, nine whole genome sequencing). The "gene test" sections and free text entries within the RaDaR database were also searched to discover those who had had clinical genetic testing with results available. RaDaR consent permits access to participants' medical records, therefore the results of genetic testing undertaken by Bristol Genetics Laboratory at Southmead Hospital were checked. Bristol Genetics Laboratory offers clinical genetic testing through the NHS using a next generation sequencing panel of 37 genes(2) or, more recently, 70 genes associated with nephrotic syndrome.(3) Results of genetic testing at other UK locations were also available for some patients in the free-text entries of RaDaR. This gave a total of 271 patients.

We have estimated the percentage of paediatric steroid resistant nephrotic syndrome patients enrolled in the UK RaDaR cohort based on an annual incidence of steroid resistant nephrotic syndrome of 0.3/100,000,(4) and a paediatric population in Great Britain of approximately 13.5 million (Office of National Statistics 2016, population aged 0-17 years inclusive). In the UK RaDaR cohort, there are 195 paediatric steroid resistant nephrotic syndrome patients diagnosed in the seven-year period between 2010 and 2016 (inclusive). We would expect there to be 7 x 0.3/100,000 x 13,500,000 = 284 cases during this period. Therefore approximately 195/284 = 69% of patients are enrolled in the RaDaR cohort. Clearly this is an

estimate only, but is as accurate as possible based on the reported data available at this time.

Data Retrieval

Demographic, clinical and long-term outcome data were extracted from the RaDaR database. The RaDaR database current to January 2018 was downloaded into Microsoft Excel and filtered on the 271 patients included in this study. The medication fields including the name of the drug, start and stop dates were extracted. In order to avoid missing treatments which had not been entered in the correct sections of the registry, a search was conducted on free text sections of the database which in many cases include anonymised copies of patient clinic letters (Supplemental Table 1). The search also used the associated medication proprietary names, common abbreviations (e.g. MMF) and alternative spellings (e.g. cyclosporin). Since the aim of this study was to examine the disease-modifying anti-proteinuric effect of the medications, they were filtered to include only those with a start date prior to the date of onset of kidney

failure. The start of kidney failure was taken as the first date with estimated glomerular filtration rate (eGFR) persistently <15ml/min/1.73m^2 or the start of renal replacement therapy, whichever was earlier. eGFR was calculated using the Schwartz formula from the plasma creatinine and patient height. For each patient, the medications were sorted by chronological order of start date. For intensified immunosuppression drugs, only the first course of each drug was included for analysis. Since the evaluation of response used a window of six months after the start date, in the case of Rituximab this may include several intravenous doses. For angiotensin-converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARB), individual drugs were considered by class and only the first course within each class was included for analysis. For example, in a patient who first received enalapril and was later changed to lisinopril, only the response to enalapril would be evaluated.

Management of missing medication data

The list of all patients receiving at least one ACEi/ARB or intensified immunosuppression prior

to kidney failure was compared with the total list of 271 patients. The RaDaR data for those appearing not to be receiving any of these medications was reviewed in detail. In some cases, a reason for the lack of medication became evident including congenital nephrotic syndrome, kidney failure at presentation or syndromic/familial steroid resistant nephrotic syndrome. If no explanation became obvious, the research teams at local recruiting centres were contacted for more information.

Analysis of response to medication

The primary outcome was defined using the change in plasma albumin and proteinuria before and within six months after starting the medication. Complete response was defined as urine protein:creatinine ratio (UPCR) <200 mg/g or negative/trace dipstick proteinuria within six months of starting therapy. Partial response was defined as UPCR >200 mg/g or dipstick ≥1+ but plasma albumin >2.5 g/dL within six months of starting therapy. In cases where the plasma albumin was already >2.5 g/dL prior to starting treatment but remained above this in the following six months and proteinuria did not reach the threshold for complete response, this was classed as partial response.

The following laboratory data were extracted from the RaDaR database for all patients who received the medications under investigation:

- Plasma albumin
- UPCR
- Urine dipstick protein
- Urine albumin:creatinine ratio (UACR)

If all measures of proteinuria were available, preference was given to UPCR. If only UACR was available, a value <30mg/g was considered equivalent to UPCR <200mg/g.

In the cases where the RaDaR record was electronically linked to laboratory data via Renal Patient View, complete results were available. In some cases, however, only limited laboratory

data were available which had been entered manually by the research teams. The data closest to, and prior, to the medication start date were taken as the baseline. Where complete results were available, the lowest UPCR and highest plasma albumin achieved together were used to judge against the criteria for complete and partial response. In some cases, only single results were available during the time frame.

If a medication was stopped within six months of starting, only laboratory data while receiving the medication were used to judge response, except in the cases of Rituximab and intravenous Cyclophosphamide which are given as intermittent doses rather than daily. If two medications were started simultaneously or within one month of each other, the same response outcome was assigned to both although it was not possible to determine which of the two, or the combination, was responsible for any improvement.

Management of missing medication response data

After completion of the above analysis, the medications for which a response could not be assigned were identified. In all cases this was due to incomplete laboratory data. Research teams at recruiting centres were approached to provide the relevant missing laboratory results. In addition, they were given an option to complete a spreadsheet listing their patients and ACEi/ARB and intensified immunosuppression medications which they were invited to complete to indicate which medications they had received and the response using the same criteria for complete and partial response as above. In order to maximise medication response completeness, free text entries in RaDaR in the period after the start date were reviewed both for laboratory results and the clinician opinion. If laboratory data alone, sought in a variety of ways, were insufficient to make a judgement on medication response, the overall clinician opinion and statements such as “absence of proteinuria” or “in remission” were used to assign a response.

Data analysis

The proportions of patients achieving complete and partial response for each medication were calculated for the cohort as a whole and stratified by genetic/non-genetic disease and by pattern of steroid resistance. Patients with non-genetic disease who suffer post-transplant recurrence represent those most likely to have a pathogenic circulating factor. The response to intensified immunosuppression medications was examined particularly in this subgroup. Since clinicians often use intensified immunosuppression drugs in a particular sequence, some drugs are used more often only after failure of others in patients who are then considered more “resistant”. To attempt to avoid this bias, outcomes for only the first intensified immunosuppression drug used per patient were analysed.

Statistical analysis

Data were analysed using Microsoft Excel 2013 and GraphPad Prism 7. Comparisons for proportions between cohorts and groups with data in 2 × 2 contingency tables used Fisher’s

exact test. Comparisons in larger contingency tables used the chi-squared test. Groups were pooled if any expected frequencies were <5. Comparisons of continuous data between the groups used the Mann-Whitney U Test. Progression to kidney failure in different subgroups was assessed using the Kaplan-Meier survival method. Analysis of differences between survival curves was by the log-rank (Mantel-Cox) test. All tests were two-tailed and $p \leq 0.05$ was considered significant.

Methodology for the Paris cohort

Data retrieval

Data were extracted from the steroid resistant nephrotic syndrome/Necker Dr Warehouse Database.(5) Between 2001 and 2019, we established a cohort of 2483 patients (from 2124 unrelated families) with primary FSGS and/or steroid resistant nephrotic syndrome diagnosed during childhood or adulthood. Patients were recruited through adult and paediatric nephrology departments in France, and blood samples were addressed to our reference centre. The cohort was approved by the Comité de Protection des Personnes Ile-De-France II. We obtained pedigree information and clinical data using a standardized questionnaire. Familial data were collected by clinicians in charge of patients, including data on consanguinity, number of affected and nonaffected siblings, and clinical data for the father and mother (proteinuria, hematuria, nephrotic syndrome, and/or chronic renal failure), based on the information provided by the families.

Next-generation sequencing and mutations filtering

Blood samples were collected after receiving written informed consent from the patients. Genomic DNA was extracted from peripheral blood by standard methods. We previously used a two-step screening algorithm for sporadic late-onset steroid resistant nephrotic syndrome, limited to the search of carriers of the p.Arg229Gln polymorphism and full sequencing of the *NPHS2* gene if positive.(6) For this study, all children with early-onset

steroid resistant nephrotic syndrome, all patients with familial steroid resistant nephrotic syndrome and all patients with sporadic late-onset steroid resistant nephrotic syndrome not carrying the *NPHS2* mutation associated with the p.Arg229Gln polymorphism were then screened using our next-generation sequencing targeted panel.(7) Thirty-five genes (655 coding exons and splice junctions) were screened using a custom targeted amplicon-based multigene next-generation sequencing panel (Multiplicom, Niel, Belgium). High-throughput sequencing was performed using a MiSeq/HiSeq platform (Illumina, San Diego, CA). Sequence alignment and downstream processing was carried out as already described. Variants were annotated and analysed using the Polyweb software interface designed by the Bioinformatics platform of Paris Descartes University, Paris, France. All pathogenic variants were verified by Sanger sequencing, which was also used when parent DNA was available. All the variants identified were evaluated to determine their pathogenic character according to the American College of Medical Genetics and Genomics guidelines for clinical sequence interpretation.(6) We used the openly available online tool for the interpretation of sequence variants described by Kleinberger et al. We considered only variants located in coding exons and essential splice site regions. Then we excluded silent mutations and splice variants that did not affect the splice site scores and all the variants present in the Exome Aggregation Consortium database with a minor allele frequency >0.01 (at least in one population). We screened missense variants with the three most commonly used bioinformatic predictors of variants' pathogenicity, namely PolyPhen-2,(8) SIFT,(9) and Mutation Taster(10) to detect highly deleterious mutations. We considered pathogenic nonsense, frameshift, essential splice, previously reported mutations (Human Gene Mutation Database professional),(11) and missense variants with high prediction scores.

Inclusion and exclusion criteria for the RaDaR cohort of idiopathic nephrotic syndrome

From its inception in 2011, the RaDaR idiopathic nephrotic syndrome cohort included children with steroid resistant nephrotic syndrome. Inclusion and exclusion criteria were as follows.

Inclusion:

- <19 years at age of onset
- Idiopathic nephrotic syndrome (nephrotic range proteinuria and hypoalbuminaemia) with failure to respond to four weeks of high-dose oral prednisolone, including
    - Congenital nephrotic syndrome (presumed steroid resistance)
    - Steroid resistant nephrotic syndrome with primary steroid resistance
    - Steroid resistant nephrotic syndrome with secondary steroid resistance
    - Nephrotic syndrome as part of a syndrome, for example Nail-Patella syndrome or Denys-Drash syndrome
- Nephrotic syndrome with focal segmental glomerulosclerosis (FSGS) on biopsy

Exclusion:

- ≥19 years at age of onset
- Nephrotic syndrome secondary to any other condition, including
    - IgA nephropathy
    - Membranoproliferative glomerulonephritis / C3 glomerulopathy
    - Membranous nephropathy
    - Vasculitis
    - Systemic lupus erythematosus
    - Hypertension
    - Obesity

- Diabetes mellitus

Following an amendment to the study protocol in December 2015, inclusion criteria were broadened to encompass patients with onset of disease at any age and all forms of idiopathic nephrotic syndrome including steroid sensitive, frequently relapsing and steroid dependent nephrotic syndrome.

Supplemental Table 1: Terms used for free-text medication search

ACEi/ARB	Immunosuppressive drugs
ACEi	Abatacept
ARB	ACTH
Captopril	Azathioprine
Enalapril	Ciclosporin
Irbesartan	Cyclophosphamide
Lisinopril	Levamisole
Losartan	Mycophenolate mofetil
Ramipril	Rituximab
Valsartan	Tacrolimus

The search also used the associated medication proprietary names, common abbreviations (e.g. MMF) and alternative spellings (e.g. cyclosporin). ACEi, angiotensin-converting enzyme inhibitor; ACTH, adrenocorticotropic hormone; ARB, angiotensin II receptor blocker.

Supplemental Table 2: 'Other' findings on kidney biopsy

Biopsy finding	Number of patients
Mesangial proliferation	4
Focal global glomerulosclerosis	2
C1q nephropathy	2
Diffuse mesangial sclerosis	1
Collapsing glomerulosclerosis	1
Alport's disease	1
Thin membrane disease	1
IgA nephropathy	1
Unspecified	1

'Other' findings on initial biopsy (excluding the three most common diagnoses of focal segmental glomerulosclerosis, minimal change disease and mesangial hypercellularity). These findings are taken from the biopsy reports uploaded onto the RaDaR database or, where unavailable, from documentation in clinic letters.

Supplemental Table 3: Correlation of patient RaDaR numbers with previously published ID numbers

RaDaR number	Previous ID
495	37 ^
514	Not previously published
687	42 + ^
729	13 ^
731	180 ^ *
770	32 ^
811	18 ^
900	21 ^
7656	Not previously published

^ Previously published in Bierzynska A, McCarthy HJ, Soderquest K, Sen ES, Colby E, Ding WY et al: Genomic and clinical profiling of a national nephrotic syndrome cohort advocates a precision medicine approach to disease management. *Kidney Int* 91(4): 937-947, 2017.

+ Previously published in Ebarasi L, Ashraf S, Bierzynska A, et al: Defects of CRB2 cause steroid-resistant nephrotic syndrome. *A J Human Genet* 96: 153-161, 2015.

* Previously published in Bierzynska A, Soderquest K, Dean P, Colby E, Rollason R, Jones C et al: MAGI2 Mutations Cause Congenital Nephrotic Syndrome. *J Am Soc Nephrol* 28(5): 1614-1621, 2017.

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