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Title: Response to first course of intensified immunosuppression in genetically-stratified steroid resistant nephrotic syndrome

Running title: Immunosuppression response in steroid resistant nephrotic syndrome

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Abstract

Background and objectives

Intensified immunosuppression in steroid resistant nephrotic syndrome is broadly applied, with disparate outcomes. This review of patients from the UK NephroS cohort aimed to improve disease stratification by determining, in comprehensively genetically-screened steroid resistant nephrotic syndrome patients, if there is an association between response to initial intensified immunosuppression and disease progression and/or post-transplant recurrence.

Design, setting, participants, and measurements

Paediatric steroid resistant nephrotic syndrome patients were recruited via the UK RaDaR registry. All patients were whole genome, whole exome or steroid resistant nephrotic syndrome-gene-panel sequenced. Complete response or partial response within six months of starting intensified immunosuppression was ascertained using laboratory data. Response to intensified immunosuppression and outcomes were analysed according to genetic testing results, pattern of steroid resistance and first biopsy findings.

Results

Of 271 patients, 178 (92 male, median onset age 4.7 years) received intensified immunosuppression with response available. 4% of monogenic disease patients showed complete response, compared to 25% of genetic-testing negative patients (p=0.02). None of the former recurred post-transplantation. In genetic-testing negative patients, 97% with complete response to first intensified immunosuppression did not progress whereas 44% of non-responders developed kidney failure with 73% recurrence post-transplant. Secondary steroid resistance had a higher complete response rate than primary/presumed resistance (43% vs 23%, p=0.001). Highest complete response rate in secondary steroid resistance

was to Rituximab (64%). Biopsy results showed no correlation with intensified immunosuppression response or outcome.

Conclusions

Patients with monogenic steroid resistant nephrotic syndrome had a poor therapeutic response and no post-transplant recurrence. In genetic-testing negative patients, there was a clear association between response to first intensified immunosuppression and long-term outcome. Patients with complete response rarely progressed to kidney failure, whereas non-responders had poor kidney survival and a high post-transplant recurrence rate. Patients with secondary steroid resistance were more likely to respond, particularly to Rituximab.

Introduction

Approximately 10-15% of children with nephrotic syndrome are resistant to steroids,(1, 2) and most of these receive intensified (or second-line) immunosuppression. Response is often disappointing and there are significant side effects. 30-40% progress to kidney failure within 10 years, requiring dialysis and transplantation.(3) Disease recurrence is common and associated with poor long-term outcome. Unfortunately, our ability to predict the disease course, treatment response and risk of post-transplantation recurrence for individual patients is limited.

There are many proposed risk factors for post-transplantation recurrence including age at diagnosis, rate of progression to kidney failure, biopsy result, ethnicity and previous recurrence.(4, 5) However, the most informative factors remain secondary steroid resistance for increased risk,(6) or a monogenic cause of disease as a protective feature. Mutations have been identified in over 70 genes, causing podocyte defects, and are responsible for approximately 30% of childhood steroid resistant nephrotic syndrome.(7-21) Patients with

genetic disease are usually resistant to immunosuppression and progress more rapidly to kidney failure but do not recur after transplantation.(7, 22)

Post-transplantation recurrence is thought to be immune-mediated. It is hypothesised that a plasma circulating factor, derived from immune cell dysfunction, acts on the podocyte and disrupts glomerular permeability. However, its identity remains elusive.(23-25) . We have previously shown that secondary steroid resistance can be used as a marker for circulating factor disease and is associated with a high risk of post-transplantation recurrence.(6) Strikingly, 93% of patients with secondary steroid resistance recurred post-transplantation compared to 30% with primary steroid resistance.

The latter study lacked detailed genetic analyses. We propose that comprehensively genetically-stratifying steroid resistant nephrotic syndrome patients, then correlating response to intensified immunosuppression with progression to kidney failure and recurrence, will identify subgroups which are useful for clinical prognostication and management. Our results identify two distinct groups of genetic-testing negative patients: one that responds to intensified immunosuppression and has a good long-term outcome, and one that is multi-drug resistant with rapid progression, very poor kidney survival and high post-transplant recurrence risk.

Materials and Methods

Patient Cohort

Cases were taken from the UK Renal Rare Disease Registry (RaDaR), a Renal Association initiative set up in 2010 which collates clinical data from patients with rare kidney diseases.(26) Data are collected both retrospectively and prospectively via an online portal and include demographics, family history, consanguinity, pattern of steroid resistance, medications, transplantation and recurrence. Cases were selected in January 2018, at which

point there were 2457 patients with idiopathic nephrotic syndrome enrolled (see supplemental material for inclusion/exclusion criteria). Patients included in this analysis had steroid resistant nephrotic syndrome with age of onset <18 years and were screened for disease causing mutations. Steroid resistant nephrotic syndrome is defined as failure to respond to four weeks of high-dose oral prednisolone. The cohort consisted of 271 patients (Figure 1) with a date of diagnosis ranging from 1995 to 2017. 188 patients had whole exome sequencing,(7) 9 whole genome sequencing, and 74 clinical gene panel testing. 68 clinical gene panel testing patients underwent testing at Bristol Genetics Laboratory with next generation sequencing of 37 (27) or 70 genes associated with steroid resistant nephrotic syndrome.(28) The remaining six clinical gene panel testing patients had testing in other locations, with results documented in RaDaR. Patients were considered to have monogenic disease if a mutation was found in one of the known 'nephrotic' genes.(7, 27) Follow-up data were inputted to the RaDaR registry on at least a six monthly basis. Local clinical teams were contacted individually to provide specific items of missing data.

Clinical Data Retrieval

Demographic, clinical and long-term outcome data were extracted from the RaDaR database. Only medications started prior to kidney failure, and only the first course of each medication, were included. Complete response was defined as urine protein:creatinine ratio (UPCR) <200mg/g, urine albumin:creatinine ratio (UACR) <30mg/g or negative/trace dipstick proteinuria within six months of starting therapy. Partial response was defined as UPCR >200mg/g or dipstick ≥1+ but plasma albumin >2.5g/dL. If a medication was stopped within six months, only laboratory data while receiving the medication were used. If two medications were started simultaneously or within one month, the same response outcome was assigned to both. For management of missing data, see supplemental material.

Data Analysis

Proportions of patients achieving complete and partial response were calculated for the whole cohort and stratified by genetic disease, pattern of steroid resistance and biopsy results. Particular attention was given to genetic-testing negative patients with post-transplant recurrence as they are most likely to have circulating factor disease. To minimise bias from the order in which clinicians chose to use medications, outcomes for the first intensified immunosuppression drug used per patient were analysed separately.

Statistical Analysis

Data analysis was performed using GraphPad Prism 7 with Fisher's exact test, Chi-squared analysis or Mann-Whitney U test.

Results

Patient Characteristics

Demographic features of the 271 patients and the treatments received are shown in Tables 1 and 2. In total, 186 patients (69%) received intensified immunosuppression. Completeness of response data was 91%. 346 intensified immunosuppression treatments with responses available were given to 178 patients.

Response and Outcomes in Patients with Monogenic Disease

Of the 271 patients, 81 (30%) had monogenic disease. 26 (32%) of these were treated with intensified immunosuppression. Complete response to first intensified immunosuppression was seen in 4% of monogenic patients compared to 25% of genetic-testing negative patients ($p=0.02$; no data n=0/26 monogenic, n=3/152 genetic-testing negative patients). There was a significant difference in combined complete and partial response between monogenic and genetic-testing negative patients for all intensified immunosuppression treatments (35% vs 53%, $p=0.04$; no data n=5/45 monogenic, n=29/335 genetic-testing negative treatment episodes) but not for first intensified immunosuppression only (35% vs 46%, $p=0.29$). One

monogenic patient had complete response and eight showed partial response to first-administered intensified immunosuppression (Table 3). None of the 21 monogenic patients (26%) who received a transplant had responded to intensified immunosuppression and none recurred post-transplantation. This is significantly different to transplanted genetic-testing negative patients who had a 68% recurrence rate (21/31) post-transplantation.

Response to Immunosuppression in Genetic-testing Negative Patients

Of the 190 genetic-testing negative patients, 152 received 306 intensified immunosuppression treatments with response data available (no data n=29/335 treatment episodes). The average number of treatments was two per patient (range one to five). Overall, the complete response rate was 28%. Complete response was highest for Rituximab (39%, 16/41; no data n=1) and lowest for cyclophosphamide (19%, 8/43; no data n=5) but this was not statistically significant (p=0.05). The combined complete and partial response rate was highest for tacrolimus (59%, 48/82; no data n=4) and this was significant compared to cyclophosphamide (40%, p=0.04, Figure 2a). Response data were available for first-administered intensified immunosuppression in 149 patients (no data n=3). Ciclosporin was the first treatment in 66 patients (44%), tacrolimus in 35 (23%) and cyclophosphamide in 32 (21%). Ciclosporin and tacrolimus had similar levels of complete response (27% and 31% respectively, Figure 2b). This was higher than cyclophosphamide (13%) but not statistically significant (p=0.13 vs ciclosporin, p=0.08 vs tacrolimus).

Association between Response to First Intensified Immunosuppression and Likelihood of Kidney Failure in Genetic-testing Negative patients

Characteristics and long-term outcomes for genetic-testing negative patients stratified by response to first intensified immunosuppression treatment are shown in Table 4. The median follow-up time was 5.2 years (range 0.1-22.2 years). Strikingly 97% of patients (36/37) with complete response showed no progression to kidney failure (median follow-up 5.0 years, range 0.1-15.2 years) (Figure 3). Non-responders had significantly quicker

progression to kidney failure as shown in the Kaplan-Meier survival curves (p<0.001, Mantel-Cox test) (Figure 2c). The five-year kidney failure-free survival rates were 97%, 87% and 59% for patients with complete, partial and no response respectively. The corresponding 10-year rates were 97%, 74% and 27%. Frequency of transplantation was significantly higher in non-responders (p<0.001). In total, 31 genetic-testing negative patients received a transplant. 26 (84%) were non-responders to first intensified immunosuppression, four had partial response and only one complete response. The overall recurrence rate was 68% (21/31) with 73% (19/26) recurrence in non-responders. Strikingly, kidney survival for genetic-testing negative patients with no response to first intensified immunosuppression is the same as for patients with monogenic disease (Figure 2c). This supersedes previous data where comparison between monogenic and (all) non-monogenic steroid resistant nephrotic syndrome shows worse kidney survival for monogenic patients.(2, 7)

Response Stratified by Pattern of Steroid Resistance

Genetic-testing negative patients with primary steroid resistance (120 patients) were analysed as a distinct subgroup from secondary steroid resistance (32 patients). Secondary steroid resistant patients had significantly higher complete response than those with genetic-testing negative primary steroid resistance (43% vs 23%, p=0.001; no data n=29/334 treatment episodes). The combined complete and partial response rate was also significantly higher (65% vs 48%, p=0.01). The highest complete response rate was to Rituximab with 64% (9/14, no data n=1) in secondary steroid resistant patients compared to 26% (7/27) in genetic-testing negative primary steroid resistance (p=0.02). Secondary steroid resistant patients also had a significantly higher complete response rate to cyclophosphamide (38% vs 10%, p=0.04; no data n=3/16 secondary steroid resistant, n=2/32 primary steroid resistant patients). When considering only first intensified immunosuppression, there was no significant difference in response between the two groups (complete response 36% vs 22%, p=0.16; combined complete and partial response 61% vs 42%, p=0.07; no data n=3/152). 22% of genetic-testing negative primary steroid resistant

patients received a transplant, with 69% (18/26) recurrence rate. 16% of secondary steroid resistant patients were transplanted with 60% (3/5) recurrence rate.

Characteristics and Outcomes in Patients Treated with Rituximab

Complete response to intensified immunosuppression was generally below 30%. The exception was Rituximab in patients with secondary steroid resistance (64%) so these patients have been analysed in more detail (Figure 4). In total, 46 patients received Rituximab. Treatment was performed according to centre-based decisions. Six were simultaneously treated with other intensified immunosuppression (one mycophenolate mofetil, one ciclosporin, three tacrolimus, one with both tacrolimus and mycophenolate mofetil). These six patients all had primary steroid resistance and showed a varied response (one complete, three partial and two non-responders). Four monogenic patients received Rituximab, and none responded. 42 genetic-testing negative patients were given Rituximab (no data n=1). Complete response was 39% (16/41) and partial response 12% (5/41); not statistically significant compared to other medications. No complete responders progressed to kidney failure (0/16, median follow-up 7.6 years). 50% (10/20) of non-responders developed kidney failure (median follow-up 5.4 years) with an 83% (5/6) post-transplant recurrence rate. There was no significant difference between responders and non-responders in terms of median age of diagnosis or time between diagnosis and treatment. 15 secondary steroid resistant patients received Rituximab (no data n=1). Complete response occurred in 64% (9/14) and none of these developed kidney failure (median follow-up 7.2 years). Of the four non-responders, two developed kidney failure and both recurred following transplantation. 27 genetic-testing negative primary steroid resistant patients received Rituximab. Complete response occurred in 26% (significantly lower than in secondary steroid resistant patients, p=0.02) and no response in 59%. 50% of non-responders developed kidney failure and three out of the four transplanted patients suffered recurrence.

To validate these findings, we examined the comprehensive database of Necker Hospital, Paris, for outcomes of paediatric steroid resistant nephrotic syndrome patients treated with Rituximab.(29) In 82 genetic-testing negative primary steroid resistant patients with no response to first intensified immunosuppression treatment, 60/82 (73%) showed no response to Rituximab. 29/60 (48%) of these developed kidney failure and 8/17 (47%) who were transplanted suffered disease recurrence. None of the 22 patients with complete (10) or partial response (12) progressed to kidney failure. No patients with secondary steroid resistance were treated with Rituximab.

Response Stratified by First Biopsy Findings

170 patients receiving intensified immunosuppression had biopsy results available (not biopsied/no data n=16). Focal segmental glomerulosclerosis (FSGS) was seen in 100 (59%) first biopsies, minimal change disease (MCD) in 45 (26%) and mesangial hypercellularity in 11 (6%). Other findings are detailed in supplemental material (Table S2). There was no significant difference in response to intensified immunosuppression or clinical outcome based on biopsy findings. When considering only FSGS and MCD, there was no significant difference in complete response (24% vs 27%, p=0.66) or in combined complete and partial response (49% vs 51%, p=0.70; no data n=27/300 treatment episodes).

Discussion

Our ability to predict long-term outcome and risk of post-transplantation recurrence in paediatric steroid resistant nephrotic syndrome is limited. There is now emerging literature regarding the differences between responders and non-responders to intensified immunosuppression.(1, 2) However, these studies lack comprehensive genetic stratification and their clinical message has not yet been widely appreciated. We present data from a large, national cohort of paediatric steroid resistant nephrotic syndrome patients with an emphasis on full genetic screening. This allows us to reinforce and validate the findings of

recent literature regarding response to intensified immunosuppression, and also adds substantial new information regarding clinical outcomes, individual therapies, biopsy findings and post-transplantation recurrence.

We focused our stratification on complete versus no response, as partial response is more susceptible to natural variation, incomplete recording, and confounding by haemodynamic factors (e.g. ACE inhibition). Nevertheless, it is interesting that most partial responders had good outcomes, suggesting they are in the same or similar immune mechanistic category as complete responders. We defined complete response as occurring within six months of starting treatment. To a certain extent, this is arbitrary and may underestimate later responders. One German study of 231 steroid resistant nephrotic syndrome patients reported 60% complete response to ciclosporin A in genetic-testing negative patients, with 18% of those achieving complete response doing so beyond six months.(30) However, the response rate in this cohort remains high even when late responders are accounted for; approximately 49% showed complete response within six months compared to 28% in our study. Our findings are very similar to that shown by the largest steroid resistant nephrotic syndrome cohort to date, where complete response to calcineurin inhibitors was 29.9%, and fits within the wider literature.(2, 31-33) We believe the six month period will capture the majority of responders whilst minimising the potential for confounding.

Our results confirm that monogenic disease is a distinct subgroup which responds very poorly to immunosuppression. There is no convincing evidence of complete response to intensified immunosuppression in monogenic disease, albeit several reported cases.(22, 30, 34) Here, one monogenic patient (out of 26) demonstrated complete response. This patient has a *WT1* mutation, with Denys-Drash Syndrome. Case reports suggest response to immunosuppression, usually cyclosporin A, in *WT1*-associated nephrotic syndrome.(35-37) Therefore, in this specific mutation, we cannot rule out a direct effect of intensified immunosuppression on podocytes. None of the 21 transplanted monogenic patients suffered

post-transplantation recurrence, compared to 68% (21/31) of genetic-testing negative patients. This is consistent with previous literature reporting that monogenic patients do not generally recur after transplantation. Patients were considered to have monogenic disease only if a mutation was found in a known ‘nephrotic’ gene, regardless of family history. Of the genetic-testing negative patients who received intensified immunosuppression, seven had an affected first-degree relative. One had presumed steroid resistance, family consanguinity (no family history) and a variant of unknown significance in *WT1*.(7) It is possible that some of these patients may have monogenic disease due to currently undiscovered gene mutations.

The genetic-testing negative subgroup is more heterogenous making it harder to predict treatment response and outcome. There was no significant difference in response or clinical outcome based on first biopsy finding, suggesting this is a poor discriminator. However, there was a clear association between response to first intensified immunosuppression and long-term outcome: responders (complete and partial) rarely developed kidney failure, whereas non-responders had a high likelihood of kidney failure and post-transplantation recurrence.

These results are generally consistent with those published from the PodoNet registry, where 27% of non-genetic patients responded completely to intensified immunosuppression, though only 43% of the cohort had gene panel sequencing, and correlation between intensified immunosuppression and post-transplant recurrence was not performed (1, 2). Our study only included patients with comprehensive genetic screening, thereby reflecting current clinical practice. 97% of patients with complete response did not develop kidney failure and the ten-year kidney failure-free survival rate was 97%. The one patient with complete response who developed kidney failure was steroid sensitive at initial treatment (secondary steroid resistance), and treated with ciclosporin. They suffered post-transplant recurrence. In contrast, 44% of non-responders to first intensified immunosuppression

progressed to kidney failure (ten-year kidney failure-free survival 28%) and the post-transplantation recurrence rate was 73%. One advantage of thorough stratification is that we show the time to kidney failure in the non-responders is as rapid as the monogenic group (Figure 2c). This is in contrast to the previous less stratified studies by ourselves and Trautmann *et al*, where monogenic patients more rapid progression than non-monogenic patients.(2, 7)

We have previously shown that secondary steroid resistance is associated with post-transplantation recurrence. In this study, there was no difference in recurrence between secondary steroid resistance and genetic-testing negative primary steroid resistance, but the numbers are too small to be conclusive. The most striking difference was the response to Rituximab. 64% of secondary steroid resistant patients showed complete response, compared to 26% (12% Paris cohort) in genetic-testing negative primary steroid resistance ($p=0.02$). No patients with complete response to Rituximab progressed to kidney failure (0/16 United Kingdom (UK) cohort; 0/10 Paris). The number of Rituxumab treated secondary steroid resistant patients is currently small but it raises an interesting new observation that should be explored further as the numbers in these cohorts increase. The clinical implication in our view is that all genetic-testing negative patients resistant to their first intensified immunosuppression should be treated with Rituximab, as a proportion (26% of genetic-testing negative primary steroid resistance, 64% of secondary steroid resistance) will respond and not progress. This is also consistent with currently 11 published observational studies and one randomised controlled trial for multidrug resistant steroid resistant nephrotic syndrome, where in total approximately 30% of almost 200 treated patients achieved complete remission.(38-49)

Some medications in secondary steroid resistant patients may have commenced whilst they were still steroid sensitive or already in remission. Our response criteria do not distinguish these patients, and this could contribute to the high response rate to Rituximab in the

secondary steroid resistance group. Of the 14 secondary steroid resistant patients receiving Rituximab (with response data available), five were steroid resistant when starting treatment, four steroid sensitive and five unknown. Complete response occurred in all steroid sensitive patients and three of the five resistant patients. None of the steroid sensitive patients progressed to kidney failure (median follow-up 8.2 years).

In summary, we have identified three subgroups of steroid resistant nephrotic syndrome which likely represent mechanistically different disease (Figure 3). Firstly, patients with monogenic disease show poor or no response to immunosuppression and generally do not recur after transplantation. Secondly, genetic-testing negative patients who respond to first intensified immunosuppression rarely progress to kidney failure. Thirdly, those who fail first intensified immunosuppression usually become multi-drug resistant with high likelihood of rapid progression to kidney failure and 73% risk of post-transplantation recurrence. These patients mostly have immune-mediated circulating factor disease, given the very high rate of post-transplant recurrence. A smaller proportion will be monogenic with currently-undiscovered gene mutations. Response to Rituximab identifies a subset of patients whose disease may be B cell mediated, although the drug could also have a direct action on podocytes.(50) Response is high in secondary steroid resistant patients and associated with good long-term outcome.

We propose that stratification according to genetic testing, steroid response and response to early immunosuppression can be valuable in guiding treatment and transplantation decisions. Our data continue to support comprehensive genetic testing in all steroid resistant children. In those who test negative, immunosuppression including calcineurin inhibitors +/- combination with steroids and other medications should be used with a trial of Rituximab in non-responders. Failure indicates the patient is likely to be multi-drug resistant and the chance of progression to kidney failure and post-transplant recurrence become high.

Disclosures

M.A.S sits on Retrophin and Pfizer advisory boards and receives grant funding from UCB and Evotec AG. O.B has received consulting fees from Novartis and lecture fees from Octapharma.

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Author Contributions A.E.M and E.S.S contributed equally to the work. M.A.S and G.I.W designed and supervised the work. A.E.M, E.S.S, M.A, A.B, E.C, A.B.K, O.B and M.W acquired the data. A.E.M and E.S.S analysed the data, drafted and revised the paper. A.B completed the genetic sequencing data analysis. All authors approved the final version of the manuscript.

Supplemental Material

Table of Contents

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Figures Legends

Figure 1: Patient cohort taken from the RaDaR registry current to January 2018. Steroid resistant nephrotic syndrome includes patients with primary, presumed and secondary steroid resistance. 184 patients were part of the original whole exome sequencing cohort that has been previously described by Bierzynska *et al* 2017.(7) RaDaR, renal rare disease registry.

Figure 2: Response to intensified immunosuppression medications and kidney survival in genetic-testing negative patients. Response to all (2a) or first-administered (2b) intensified immunosuppression medications. The number of treatments with response data available is given in brackets. No data were available for 29/335 treatment episodes (first intensified immunosuppression in three patients). Kidney survival (2c) analysed by response to first intensified immunosuppression treatment. Numbers in the table represent the number of patients at risk for each time point.

Figure 3: Patient stratification and long-term outcomes according to genetic testing and response to first intensified immunosuppression. The three stratified patient groups are indicated in the grey highlighted boxes.

Figure 4: Response to Rituximab and long-term outcome according to genetic-testing and pattern of steroid resistance.

Tables

Table 1: Demographic characteristics of the patient cohort

		Total cohort	Genetic-testing negative	Monogenic disease
Total patients		271	190	81
Male (%)		137 (51)	98 (52)	39 (48)
Age at onset (years) – number (%)	0-0.25	36 (13)	8 (4)	28 (35)
	0.25-1	10 (4)	4 (2)	6 (7)
	1-5	129 (47)	107 (56)	22 (27)
	6-12	74 (27)	55 (29)	19 (24)
	13-18	22 (8)	16 (8)	6 (7)
Median age at onset (years) / Interquartile range		4.8 / 2.4 – 9.3	4.9 / 2.6 – 9.4	3.2 / 1.6 – 8.4
Family history positive / number with data available (%)		39 / 252[×] (15)	16 / 180 (9)	23 / 72 (32)
Consanguinity / number with data available (%)		25 / 245 (10)	9 / 175 (5)[∞]	16 / 70 (23)
Ethnicity (% of patients where data available)	**White**	186 (72)	130 (70)	56 (76)
	Asian	18 (7)	15 (8)	3 (4)
	Pakistani	17 (7)	10 (5)	7 (10)
	Black African / Caribbean	13 (5)	10 (5)	3 (4)
	Mixed	8 (3)	8 (4)	0 (0)
	Indian	10 (4)	7 (4)	3 (4)
	Bangladeshi	2 (1)	2 (1)	0 (0)
	Other	6 (2)	4 (2)	2 (3)
	No ethnicity data available	11	4	7
First biopsy findings (% of patients where data available)	**FSGS**	128 (55)	103 (60)	25 (40)
	Minimal change disease	56 (24)	45 (26)	11 (18)
	Mesangial hypercellularity	16 (7)	9 (5)	7 (11)
	Finnish type	6 (3)	0 (0)	6 (10)

	Kidney failure	5 (2)	0 (0)	5 (8)
	Diffuse mesangial sclerosis	4 (2)	2 (1)	2 (3)
	Focal global glomerulosclerosis	2 (1)	1 (1)	1 (2)
	Other	17 (7)	11 (6)	6 (10)
	No biopsy data available / Not biopsied	37	19	18
Pattern of steroid resistance (%)	**Presumed**	54 (20)	15 (8)	39 (48)
	Primary	179 (66)	138 (73)	41 (51)
	Secondary	38 (14)	37 (19)	1 (1)

Percentages are calculated for column totals. ˣPatients were deemed to have a positive family history if they had an affected first degree relative, or an affected cousin in a consanguineous family. In the genetic-testing negative group this includes seven siblings from three families. The monogenic disease group includes 13 siblings from six families. ᵒ°One patient from a consanguineous family had no mutations identified in known nephrotic genes but is under investigation for a novel gene candidate. FSGS, focal segmental glomerulosclerosis.

Table 2: Number of treatments received and availability of response data

Groups	Subgroups	Number of patients	Number of treatments	Number of patients with response data	Number of treatments with response data
Total cohort		271	-	-	-
Not receiving ACEi/ARB or intensified immunosuppression (%)	**Total**	52 (19)	-	-	-
Reason for no ACEi/ARB or intensified immunosuppression	**Congenital nephrotic syndrome**	24	-	-	-
	CKD/kidney failure at presentation	13	-	-	-
	Syndromic	3	-	-	-
	Familial	1	-	-	-
	No medication data	11	-	-	-
Total receiving treatments (%)	**ACEi/ARB or intensified immunosuppression**	219 / 271 (81)	540	202 / 219 (92)	480 / 540 (89)
Grouped by patients (%)	**ACEi/ARB only**	33 / 219 (15)	-	-	-
	Intensified immunosuppression only	86 / 219 (39)	-	-	-
	ACEi/ARB and intensified immunosuppression	100 / 219 (46)	-	-	-
Grouped by treatments (%)	**All ACEi/ARB**	133 / 219 (61)	160 / 540 (30)	112 / 133 (84)	134 / 160 (84)
	All intensified immunosuppression	186 / 219 (85)	380 / 540 (70)	178 / 186 (96)	346 / 380 (91)

ACEi, angiotensin converting enzyme inhibitor; ARB, Angiotensin II receptor blocker; CKD, chronic kidney disease.

Table 3: Patients with monogenic disease who responded to immunosuppression

Patient	Gene	Gender	Age at onset (years)	Resistance to steroids	1st biopsy	CKD stage	Extra-renal phenotype	Length of follow-up (years)	Medication for which there was response	Response
7656	*WT1*	M	3	primary	Diffuse mesangial sclerosis	2	Denys-Drash syndrome	1.6	MMF Tacrolimus	Complete Complete
495	*NPHS1*	F	2	primary	Minimal change disease	2	No	17.8	MMF	Partial
514	*SMARCAL1*	M	7	primary	FSGS	1	No	0.4	Ciclosporin	Partial
687	*CRB2*	F	0	presumed	Minimal change disease	1	No	4.0	Levamisole	Partial
729	*NPHS2*	M	7	primary	Other	2	Asthma	2.8	Tacrolimus	Partial
731	*MAGI2*	M	0	primary	Minimal change disease	1	Pyloric stenosis, Polydactyly, Thrombocytosis	11.7	Ciclosporin	Partial
770	*COL4A3*	F	7	primary	FSGS	1	No	2.5	Ciclosporin	Partial
811	*WT1*	F	3	primary	Alports	1	Chronic cough and diarrhoea, Frasier syndrome	1.1	Tacrolimus	Partial
900	*LMX1B*	F	14	primary	FSGS	1	Delayed puberty	1.1	Tacrolimus	Partial

CKD, chronic kidney disease; F, female; FSGS, focal segmental glomerulosclerosis; M, male; MMF, mycophenolate mofetil. For correlation of RaDaR numbers with previously published ID numbers see supplemental material (Table S3).

Table 4: Characteristics and long-term outcomes of genetic-testing negative patients stratified by response to first intensified immunosuppression treatment

		Total with outcomes	Complete	Partial	No	P Value
Number of patients		149	37	32	80	
First-line intensified immunosuppression treatment	Ciclosporin	66 (44)	18	17	31	0.53[+]
	Tacrolimus	35 (23)	11	5	19	
	Mycophenolate Mofetil	6 (4)	2	0	4	
	Cyclophosphamide	32 (21)	4	8	20	
	Rituximab	5 (3)	1	1	3	
	Levamisole	4 (3)	1	1	2	
	Azathioprine	1 (1)	0	0	1	
Age at onset in years – number (% of column total)	0-0.25	0 (0)	0 (0)	0 (0)	0 (0)	0.03[^]
	0.25-1	1 (1)	0 (0)	0 (0)	1 (1)	
	1-5	89 (60)	26 (70)	24 (75)	39 (49)	
	6-12	47 (32)	11 (30)	5 (16)	31 (39)	
	13-18	12 (8)	0 (0)	3 (9)	9 (11)	
Pattern of steroid resistance	Presumed steroid resistance	1 (1)	0 (0)	0 (0)	1 (1)	0.15[*]
	Primary steroid resistance	117 (79)	26 (70)	24 (75)	67 (84)	
	Secondary steroid resistance	31 (21)	11 (30)	8 (25)	12 (15)	
Number (%) who developed kidney failure		41 (28)	1 (3)	5 (16)	35 (44)	<0.001[&]
Number (%) transplanted		31 (21)	1 (3)	4 (13)	26 (33)	<0.001[&]
Number (% of those transplanted) with post-transplant recurrence		21/31 (68)	1/1 (100)	1/4 (25)	19/26 (73)	0.30[$]

Treatment response data were unavailable for the first intensified immunosuppression treatment in three genetic-testing negative patients (all three received ciclosporin). All data

were complete for age, steroid resistance, kidney failure, transplant and post-transplant recurrence. Percentages are calculated for column totals. p values are for the comparison between complete, partial and no response. [+] Chi-squared analysis, 8df. Azathioprine and levamisole excluded from analysis. [^] Chi-squared analysis, 4df. “0-0.25”, “0.25-1” and “1-5” combined into one group. [*] Chi-squared analysis, 2df. “Presumed” and “Primary steroid resistance” combined into one group. [&] Chi-squared analysis, 2df. [$] Fishers exact test. Complete and partial response combined into one group.