Initial Steroid Sensitivity in Children with Steroid-Resistant Nephrotic Syndrome Predicts Post-Transplant Recurrence

Wen Y. Ding,*† Ania Koziell,‡§ Hugh J. McCarthy,*† Agnieszka Bierzynska,† Murali K. Bhagavatula,† Jan A. Dudley,* Carol D. Inward,* Richard J. Coward,*† Jane Tizard,* Christopher Reid,‡ Corinne Antignac,¶ Olivia Boyer,¶ and Moin A. Saleem*†

*Department of Paediatric Nephrology, Bristol Royal Hospital for Children, Bristol, United Kingdom; †Academic Renal Unit, University of Bristol, Southmead Hospital, Bristol, United Kingdom; ‡Department of Paediatric Nephrology, Evelina Children’s Hospital, London, United Kingdom; §Department of Experimental Immunobiology, King’s College London, Guy’s Hospital, London, United Kingdom; ‖Children’s Hospital for Wales, Cardiff, United Kingdom; and ¶Pediatric Nephrology, French Institute of Health and Medical Research Unit 983, Necker Hospital for Sick Children, Paris, France

ABSTRACT

Of children with idiopathic nephrotic syndrome, 10%–20% fail to respond to steroids or develop secondary steroid resistance (termed initial steroid sensitivity) and the majority progress to transplantation. Although 30%–50% of these patients suffer disease recurrence after transplantation, with poor long-term outcome, no reliable indicator of recurrence has yet been identified. Notably, the incidence of recurrence after transplantation appears reduced in patients with steroid-resistant nephrotic syndrome (SRNS) due to monogenic disorders. We reviewed 150 transplanted patients with SRNS to identify biomarkers that consistently predict outcome of SRNS after transplantation. In all, 25 children had genetic or familial SRNS and did not experience post-transplant recurrence. We reviewed phenotypic factors, including initial steroid sensitivity, donor type, age, ethnicity, time to ESRD, and time on dialysis, in the remaining 125 children. Of these patients, 57 (45.6%) developed post-transplant recurrence; 26 of 28 (92.9%) patients with initial steroid sensitivity recurred after transplantation, whereas only 26 of 86 (30.2%) patients resistant from the outset recurred (odds ratio, 30; 95% confidence interval, 6.62 to 135.86; P<0.001). We were unable to determine recurrence in two patients (one with initial steroid sensitivity), and nine patients did not receive initial steroids. Our data show that initial steroid sensitivity is highly predictive of post-transplant disease recurrence in this pediatric patient population. Because a pathogenic circulating permeability factor in nephrotic syndrome remains to be confirmed, we propose initial steroid sensitivity as a surrogate marker for post-transplant recurrence.


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W.Y.D. and A.K. contributed equally to this work.

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Correspondence: Dr. Moin Saleem, Academic Renal Unit, University of Bristol, Second Floor, Learning and Research, Southmead Hospital, Bristol BS10 5NB, UK. Email: m.saleem@bristol.ac.uk

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Idiopathic steroid-resistant nephrotic syndrome (SRNS), interchangeably termed by the common histologic description of FSGS, is one of the commonest causes of end stage renal failure (ESRF) in childhood and children with SRNS are the most prevalent group in the US dialysis registry (comprising 14.4% of all pediatric dialysis patients).1 Approximately 50%–60% of children with SRNS eventually require a renal transplant. Unfortunately, this group of patients has a high incidence of disease recurrence, with rates as high as 50% reported.2–4 Several studies have examined possible risk factors for recurrence. To date, non–African-American race, previous recurrence after transplantation, and time <3 years to ESRF have been proposed to directly correlate with an increased risk of recurrence.5–7
Recent advances in our knowledge of the genetics of SRNS have highlighted the potential importance of gene mutations in helping to predict risk of disease recurrence, the majority of which cause structural/signaling changes at the filtration barrier.\textsuperscript{8} Specifically, a number of studies have shown that patients with autosomal recessive SRNS and either homozygous or compound heterozygous mutations in NPHS2 (which encodes podocin, a slit diaphragm protein) have very low recurrence rates of 0%–3% after transplantation.\textsuperscript{6,9,10} On the other hand, haploinsufficient and apparently sporadic individuals with only a single heterozygous NPHS2 variant (e.g., R229Q) appear to have similar recurrence rates to those with no gene mutation identified.\textsuperscript{6,10} However, the contribution of genetic background remains incompletely understood, because another study was unable to replicate these data and showed no recurrences in NPHS2 haploinsufficiency.\textsuperscript{9} Nonetheless, the true genotype may not have been recognized with the other pathogenic SRNS mutation remaining unidentified, and additionally, digenic inheritance has been described in some forms of SRNS.\textsuperscript{11} Mutations present in other key podocyte genes such as NPHS1 or WT1 are similarly associated with a low risk of disease recurrence. Patients with genetic mutations also appear to have a reduced response to immunosuppression (17% versus 68% in one study) and progress more quickly to ESRF.\textsuperscript{12}

AT cell–derived circulating factor responsible for nephrotic syndrome was initially proposed in 1974.\textsuperscript{13} To date, a number of possible candidates have been identified, including hemopexin, cardiotoxin-like cytokine 1, and soluble urokinase-type plasminogen activator receptor.\textsuperscript{14,15} Subsequent work has been supportive of this circulating factor being responsible for SRNS recurrence after transplantation.\textsuperscript{16} Because the majority of patients with podocyte gene mutations appear to be resistant to immunosuppression and do not develop recurrent disease after transplantation, and the evidence suggests that the circulating factor is immunologically derived, we hypothesized that initial steroid sensitivity is a marker for circulating factor/idio nephritic syndrome, which segregates to a higher risk of post-transplant recurrence.

For idiopathic SRNS in childhood, we analyzed the following potential risk factors: young age at diagnosis, rapid progression to ESRF, young age at transplant, positive family history, and extrarenal abnormalities that might suggest an underlying syndrome (predictive of genetic mutation) versus initial steroid sensitivity, late-onset disease, lack of family history, no evidence of extrarenal features, and lack of an identified single gene mutation. We then examined whether each factor had an increased risk of disease recurrence and whether any of these parameters could be reliably identified as risk factors for post-transplant recurrence.

**RESULTS**

We identified 150 patients transplanted for ESRF secondary to SRNS between 1981 and 2012 in three large pediatric renal transplant centers in the United Kingdom and France. Of these patients, 57 (38%) had documented post-transplant recurrence and 91 had no recurrence (60.7%). It was not possible to determine outcome in two patients because one became anuric after transplantation and died 1 month later, and the second patient developed irreversible graft thrombosis within 24 hours and required transplant nephrectomy. The first patient was initially steroid sensitive, whereas these data were unavailable for the second patient.

Patient characteristics in the recurring and nonrecurring groups are shown in Table 1. In brief, participants were classified by race as follows: 115 patients were Caucasian, 17 were North African, 6 were Afro-Caribbean, 5 were East African, 4 were Indian or Pakistani, 1 was of other Asian race, 1 was of mixed race (white and black Caribbean), and 1 was of unknown race. The median age of patients at diagnosis was 4.0 years (range, 0.3–15.5), median time to ESRF was 3.0 years (range, 0–17), and median age at first transplant was 11.5 years (range, 2.5–23.5).

The 57 patients with post-transplant recurrence had almost double the rate of complications, graft loss, and subsequent retransplantation compared with patients who did not have recurrence (28% versus 16.5%). One patient underwent three transplants; graft loss was attributed to rejection in the first two (with primary nonfunction) and recurrence in the third graft. An additional 10 patients had two transplants. Two patients had thrombosis or renal vein occlusion with the first graft, followed by disease recurrence in the second graft. The histopathologic reports of the removed grafts showed extensive necrosis in the first patient and hemorrhagic infarction in the second patient, with no evidence of recurrence in either. In eight patients, graft loss was due to disease recurrence in both transplants. Five patients lost their first graft due to recurrence and were not retransplanted. Of the 91 patients without recurrence, 15 patients lost their grafts. One patient had four transplants and 11 had two transplants; the remaining 3 patients did not have retransplants.

**Genetic Mutations and Family History**

Genetic testing (either by conventional Sanger sequencing or an extended gene panel by Next Generation Sequencing\textsuperscript{17}) in 62 of 150 patients revealed 18 pathogenic mutations. Of the 62 tested patients, 7 had pathogenic NPHS2 mutations, 4 had WT1 splicing mutations (2 with Frasier syndrome and 2 with Denys–Drash syndrome), 1 had a pathogenic NPHS1 mutation, 1 had an unbalanced chromosomal translocation between chromosomes 2 and 6, 1 had a COQ2 mutation, 1 had a PLCG1 mutation, 1 had a SMARCAL1 mutation, 1 had a TRPC6 mutation, and 1 was heterozygous for NPHS1 and heterozygous for NPHS2 mutations. None developed post-transplant recurrence. Of 62 patients, 3 carried heterozygous changes in NPHS2, but data mining using variant prediction tools and the human mutation database indicated that these were nonpathogenic. All three patients had recurrence of disease. Another patient with recurrence had a NPHS1 heterozygous variant of unknown significance.
Of 150 children (without extrarenal abnormalities/known genetic mutations), 3 had positive family histories of a first-degree relative with isolated SRNS. Two patients were siblings. An additional four children had extrarenal abnormalities with positive family histories. Two patients were siblings with Schimke's immune-osseous dysplasia, one had features of Galway Mowat syndrome, and one was dysmorphic with cardiomegaly, cataracts, and learning disabilities. None of these patients had recurrence. One patient had a distant cousin with steroid-sensitive nephrotic syndrome (SSNS) who recovered; therefore, this is more likely a chance association, rather than a positive family history, because SSNS has an increased incidence in this particular ethnic group. One patient had a brother who died at age 2 years, but the cause was unknown. There were four children with extrarenal abnormalities without a positive family history or mutation. One patient had pulmonary stenosis with infertility, one had developmental delay with epilepsy and hearing impairment, one had learning disabilities, and one had sensorineural hearing loss, primary T-cell immune deficiency, growth hormone deficiency, hypothyroidism, Addison's disease, and external ophthalmoplegia with a suspected mitochondrial cytopathy. The child with learning disabilities and no other extrarenal features had a recurrence after transplantation.

There were 25 patients with pathogenic genetic mutations (n=18) and positive family histories (n=7). None of these patients recurred after transplantation (odds ratio [OR], 0.02; 95% confidence interval [95% CI], 0.001 to 0.39; P<0.001). Because patients with genetic mutations are known to have a low risk of recurrence, we excluded these 25 patients from the subsequent analyses. We also excluded the two patients for whom recurrence could not be determined.

**Initial Steroid Sensitivity**

Initial steroid sensitivity was defined as complete remission of proteinuria (3 days of trace or negative proteinuria on dipstick testing) on at least one episode after steroid therapy. Of 28 patients with initial steroid sensitivity, 26 developed recurrent disease after transplantation, comprising 45.6% (26 of 57) of the patients who recurred. One patient had initial steroid sensitivity but became anuric immediately after transplantation and never recovered function. Although delayed graft function is a common presentation of early recurrence, we did not include this patient as a definite recurrence because the presence of proteinuria could not be established. In 9 of 123 children, steroids were either not used initially (n=3) or these patients were in ESRF at presentation (n=6). Our results showed that 26 of 28 patients (92.9%) with initial steroid sensitivity recurred after transplantation, whereas only 26 of 86 (30.2%) of those resistant from the outset recurred. Initial steroid sensitivity at presentation was an accurate and highly significant risk factor for recurrence (OR, 30; 95% CI, 6.62 to 135.86; P<0.001) (Figure 1, Table 2). None of these patients had extrarenal abnormalities.

We also performed a subgroup analysis of those patients who had genetic testing and were found to have no genetic variants. This group comprised 41 patients, 37 of which received steroids initially. Of the 37 patients, 9 were initially steroid sensitive and 28 were steroid resistant. Recurrence was higher in the initially steroid-sensitive group (8 of 9; 88.9%) compared with the initially steroid-resistant group (15 of 28; 53.6%), but this difference was not significant (OR, 6.93; 95% CI, 0.76–63.05; P=0.06).

**Donor Type**

A small proportion of patients (11 of 123) had living donor transplants. Living donor transplants confer an increased risk of recurrence (OR, 6; 95% CI, 1.24–29.06; P=0.02).

**Ethnicity**

We compared African-European patients with non-African-European patients and found that there was no significant

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Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Recurrence</th>
<th>No Recurrence</th>
<th>Unable to Determine Recurrence</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>57</td>
<td>91</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Ratio of male patients/female patients</td>
<td>1.02:1</td>
<td>1.38:1</td>
<td>0.2</td>
<td>0.40</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td>0.13</td>
</tr>
<tr>
<td>Caucasian (European/North American)</td>
<td>66.7</td>
<td>82.4</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Caucasian (North African)</td>
<td>17.5</td>
<td>7.7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Afro-Caribbean</td>
<td>5.3</td>
<td>3.3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>East African</td>
<td>1.8</td>
<td>4.4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Indian/Pakistani</td>
<td>5.3</td>
<td>1.1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Other Asian</td>
<td>1.8</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mixed race</td>
<td>1.8</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deceased donor</td>
<td>84.2</td>
<td>94.5</td>
<td>50</td>
<td>0.05</td>
</tr>
<tr>
<td>Age at diagnosis (yr)</td>
<td>4.5 (0.7–12.5)</td>
<td>3.5 (0.3–15.5)</td>
<td>2</td>
<td>0.97</td>
</tr>
<tr>
<td>Time to ESRF (yr)</td>
<td>4 (0–17)</td>
<td>3 (0–16)</td>
<td>7</td>
<td>0.09</td>
</tr>
<tr>
<td>Age at first transplant (yr)</td>
<td>12.5 (3–23)</td>
<td>11 (2.5–23.5)</td>
<td>8.5 (7.5–9.5)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Data are presented as percentages or medians (ranges), unless otherwise indicated. *P value between the recurrence and nonrecurrence groups.
difference in recurrence rates (OR, 0.92; 95% CI, 0.24 to 3.6; P=0.99). We included Afro-Caribbean and East African patients in our African-European group.

**Age at Diagnosis, Time to ESRF, Time on Dialysis, and Age at First Transplant**

No significant difference was detected in age at diagnosis (OR, 0.88; 95% CI, 0.39 to 1.9; P=0.84), time to ESRF (OR, 0.68; 95% CI, 0.31 to 1.47; P=0.44), time on dialysis (P=0.18; OR, 0.56; 95% CI, 0.26 to 1.19), or age at first transplant (OR, 0.74; 95% CI, 0.36 to 1.53; P=0.47) (Figure 1, Table 2) between the recurrent and nonrecurrent groups.

**DISCUSSION**

Our study is the first to clearly identify a highly predictive clinical feature for post-transplant disease recurrence in SRNS, and secondarily shows that the presence of genetic disease is protective.

The mechanism remains speculative, but our data keep immune-mediated pathogenicity in the spotlight. A number of lines of evidence point to a putative circulating factor being central to the pathogenic mechanisms in SSNS as well as recurrence in post-transplant SRNS. Although a number of candidates have been proposed, the factor has not yet been conclusively identified. This concept is supported by documented high rates of recurrence in SRNS after transplantation, response to plasma exchange, as well as supportive *in vitro* data showing the deleterious effects of diseased plasma on isolated glomeruli, the slit diaphragm, the actin cytoskeleton, and podocyte motility. Furthermore, the response of cultured podocytes exposed to SRNS disease plasma from consecutive patients is consistent, indicating that this is likely to be a discrete cellular response, caused by a specific circulating factor rather than heterogeneous effects. Because there is evidence to suggest that the putative circulating factor may result from immune activation, we hypothesized that the initial response to steroids might by definition indicate the presence of a similar process and thus predict risk of recurrence after transplantation. Conversely, we predicted that steroid resistance from the outset, especially if combined with a definitive podocyte gene mutation, might be protective.

We first examined all factors including aggressive course before transplantation with a time interval between onset of the disease and CKD stage 5 of <3 years, male sex, mesangial hypercellularity in most glomeruli as well as fewer sclerotic lesions, age at diagnosis, donor type, and time to ESRF, which have been correlated with increased risk for SRNS recurring after transplantation. We were unable to draw conclusions on the relationship between mesangial hypercellularity because detailed histology was not available for all patients. None of the above factors were statistically significant in predicting recurrence except for donor type (Figure 1, Table 1). Patients who received a living donor transplant had a significant risk of recurrence in our study; however, there were few living donors in our study. Previous studies of living donors and recurrence risk have had inconsistent results. We did not differentiate between genetic and nongenetic FSGS. In addition, collapsing FSGS is also a well described risk factor. This was true for the only patient in this series with collapsing FSGS (HIV-negative), who recurred immediately after transplantation and lost graft function soon afterward (patient 3, Supplemental Material).

**Table 2. ORs for post-transplant recurrence in children with nongenetic/nonfamilial SRNS**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of diagnosis &lt;6 yr</td>
<td>0.88 (0.40 to 1.90)</td>
<td>0.84</td>
</tr>
<tr>
<td>Time to ESRF &lt;3 yr</td>
<td>0.68 (0.31 to 1.47)</td>
<td>0.44</td>
</tr>
<tr>
<td>Age of first transplant &lt;12 yr</td>
<td>0.74 (0.36 to 1.53)</td>
<td>0.47</td>
</tr>
<tr>
<td>Time on dialysis &lt;2 yr</td>
<td>0.56 (0.26 to 1.19)</td>
<td>0.18</td>
</tr>
<tr>
<td>African-European race</td>
<td>0.92 (0.24 to 3.61)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Living donor transplant</td>
<td>6.00 (1.24 to 29.06)</td>
<td>0.02</td>
</tr>
<tr>
<td>Steroid sensitivity</td>
<td>30.00 (6.62 to 135.86)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Figure 1.** Forest plot of ORs for recurrence in nongenetic/nonfamilial cases. Clinical risk was calculated for each factor listed in the left column. Calculated significance is given in Results.
Decreased risk of post-transplant recurrence has been previously linked to the presence of a genetic mutation, as well as to African-American race. African-American race has consistently been shown to decrease the risk of recurrence after transplantation. Our study demonstrates different results in our African-European population, in which no significant racial effect on recurrence was seen. There is some evidence that MYH9 risk alleles (for FSGS), which are more common in Africans, have differential frequencies across different countries, and there is the potential that these risk alleles might play a part in SRNS recurrence.

A histologic diagnosis of FSGS was traditionally thought to correspond to an increased risk of steroid resistance in childhood nephrotic syndrome. With the advent of genetic testing and the increasing rate of detection of gene mutations in SRNS, it is becoming apparent that children with monogenic gene mutations might play a part in SRNS recurrence. The presence of genetic/developmental disease was clearly protective for recurrence, because none of our patients with documented gene mutations or identifiable syndromes relapsed after transplantation. Individuals that fall into a gray area between these two phenotypes are those patients with treatment-resistant SRNS with no evidence of initial steroid sensitivity who recurred after transplantation. Notably, 31 of 57 of the patients with recurrence did not exhibit initial steroid sensitivity. This remains unexplained, although possibilities include compliance/toxicity issues or aggressive disease masking initial steroid sensitivity with subsequent resistance to other standard immunosuppression regimes. We did not analyze whether any of those 31 patients exhibited a response to more intensive immunosuppression, and clearly that would be an important parameter to follow up on in this group. On the basis of our findings, we have suggested a clinical paradigm to assist in assessing pediatric patients with progressive nephrotic syndrome who are potentially at risk of transplant recurrence (Figure 2).

In conclusion, we have detected a highly significant correlation between initial steroid-sensitive and post-transplant FSGS recurrence. We propose that this is a clinically identifiable group that is at high risk (Figure 3) and could potentially be aggressively targeted with detailed preparation and additional immunosuppression before transplantation. In addition, transplantation must be planned with extra care to maximize graft survival in these patients.

**CONCISE METHODS**

**Patient Selection**

We identified pediatric patients at the Bristol Royal Hospital for Children (Bristol, UK), the Evelina Children’s Hospital (London,
UK), and the Necker Hospital for Sick Children (Paris, France) who were diagnosed with FSGS/SRNS at between 4 months and 16 years of age and who received a renal transplant between January 1, 1981, and September 1, 2012. We conducted a retrospective data collection of clinical characteristics including sex, race, age at diagnosis, time to ESRF, age at transplant, initial steroid sensitivity at presentation, genetic mutations, family history, extrarenal abnormalities, recurrence, rejection, and graft loss.

Patients were included in the study if a positive biopsy result of FSGS was found in the patient notes (143 of 150) or, at a minimum, if SRNS was the stated diagnosis in their medical records. We excluded patients with congenital nephrotic syndrome (onset age <3 months) or diffuse mesangial sclerosis on biopsy. Proteinuria was defined by having a urine protein/creatinine ratio of >300 mg/mmol. Steroid resistance was defined as the persistence of proteinuria (>300 mg/mmol or 300 mg/dl or equivalent on dipstick testing) after 4 weeks of daily prednisolone at a dosage of 60 mg/m2 per day + 3 daily doses of intravenous methylprednisolone at 600 mg/m2. Patients were deemed to have recurred if they had persistent proteinuria of >300 mg/mmol or >1 g/d after transplantation.

Statistical Analyses
Nonparametric data were analyzed using the Mann–Whitney U test. Contingency tables were analyzed with Fisher’s exact test and ORs (with 95% CIs) were used to compare recurrence and nonrecurrence groups. P<0.05 was considered significant. Values are reported as mean±SEM or the median (range).

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DISCLOSURES
None.

REFERENCES


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