***NPHS2* V260E homozygosity is a frequent cause of sporadic steroid resistant nephrotic syndrome in Black South African children**

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**Abstract: Word count 305 (Lancet 300)**

**Background**

Black children in southern Africa with nephrotic syndrome (NS) have higher prevalence of steroid resistant (SR) NS due to focal segmental glomerulosclerosis (FSGS) than Indian or White children. Renal risk variants in *APOL1* and homozygous mutations in NPHS2 are frequently identified in children with FSGS. To determine if variants in *APOL1* or *NPHS2* contributed to disparities in steroid response and FSGS histology, we investigated unrelated children with sporadic NS.

**Methods**

Indian and Black children with NS (n=79) were enrolled from two tertiary hospitals in KwaZulu-Natal, South Africa; only SRNS children underwent kidney biopsy. DNA was available for sequencing of NPHS2 for 65 NS cases and 107 ethnicity-matched controls. A replication group comprised Black SR-FSGS (n=20) cases and age-matched controls (n=19).

**Findings**

55% of Indian and 97.4% of Black children with NS were steroid resistant

(p<10-5); FSGS was diagnosed in 72.7% Indian and 81.6% Black children with SRNS (p=0.5). Homozygosity for the *NPHS2* V260E was identified in 8/30 (26.7%) of SRNS Black children, all of whom had biopsy-proven FSGS. *NPHS2* V260E homozygosity was replicated in 6/20 (30%) children with SRNS/FSGS. No steroid sensitive (SS) cases of NS or Indian SRNS cases or controls carried V260E; 1 Black normal donor was heterozygous for V260E. Analysis of shared haplotypes inherited from individual subjects’ two parents excluded a single common ancestor within 20 generations. No association was found for *APOL1* with SRNS or FSGS.

**Interpretation**

The high rate of SR-FSGS among unrelated Black children in southern Africa is partially explained by homozygosity for V260E pathogenic mutation in 30% with biopsy-proven SR-FSGS. Testing for p.V260E in Black children with NS will identify the subset with SR-FSGS making it possible to avoid kidney biopsy or ineffective treatment modalities and will provide precision diagnosis. The high rate of *NPHS2*-associated SR-FSGS is not due to consanquinity among affected children. The history of *NPHS2* V260E suggests that this mutation may be widely dispersed among black Africans in southern Africa.

**Introduction**

Steroid resistant nephrotic syndrome is a frequent cause of end stage kidney disease in children. South African Black children have higher rates of steroid resistant nephrotic syndrome (SRNS) compared to their non-African ancestry counterparts. Nephrotic syndrome (NS) is the consequence of damage to the glomerular filteration barrier, with resultant significant proteinuria, hypoalbuminemia, edema and hyperlipidemia.1 Nephrotic syndrome presenting in childhood varies considerably in resource-limited countries compared to resource-replete countries, due to differing environmental factors, infections and ethnic origin.2-4In South Africa the most common histological form of nephrotic syndrome in Black children is focal segmental glomerulosclerosis (FSGS) while minimal change disease is most common in White and Indian children.3 Over 95% of White and Indian children with minimal change disease are steroid sensitive while only 0-5% of Black children with FSGS show response to steroid treatment.5. Indian children with steroid resistant nephrotic syndrome also respond better to oral cyclophosphamide treatment compared to Black children, who are 3-fold less likely to achieve complete remission (69% vs. 20% in Indians and Blacks, respectively).6,7 The basis for these disparities is not understood, but it is hypothesized to be due to in part to genetic differences in genes predisposing to steroid resistant (SR)-FSGS.

To date variants in 27 genes have been identified that segregate with FSGS in families or are associated with sporadic FSGS.8,9 Autosomal recessive variants tend to be highly penetrant, causing congenital or childhood onset SR-FSGS, in contrast to autosomal dominant muations which tend to be associated with later onset and milder disease course.10 Childhood SRNS/FSGS has been most frequently associated with mutations in the *NPHS1* and *NPHS2* genes, which are essential for the maintenance of the podocyte ultrastructure and the podocyte slit diaphagm.11 *NPHS1* encodes nephrin, a transmembrane protein that serves structural and signaling functions critical for podocyte function.8,12,13; although *NPHS1* mutations are most often associated with congential nephrotic syndrome, there is a spectrum of age of onset depending in part of the pathogenicity of the causal mutation.14,15 *NPHS2* encodes the transmembrane protein podocin, which recruits nephrin to lipid rafts in the plasma membrane, essential to maintaining the integrity of the podocyte slit diaphragm.13,16,17 Pathogenic mutations in *NPHS2* are the most common genetic cause of SRNS/FSGS in childhood, generally associated with disease onset from about 3 months to 9 years of age.18-20

The association of monogenic mutations differs for familial and sporadic forms of NS as well as among different racial and ethnic groups. For familial forms of SRNS, identification of single gene causes for any of the known 27 NS genes is inversely correlated to age of onset and positively correlated to the degree of consanquinity.21 In a study of 1783 families undergoing mutational analysis for NS genes, overall a single gene cause of SRNS was found for 29.5% of families and up to 45% of consanquinous families.21 In contrast, recent USA studies of sporadic pediatric SRNS suggest that monogenic causes are infrequently detected. *NPHS2* mutations are observed in 5-10% of White children, due primarily to the European founder mutations, *NPHS2* p.R138Q and p.R229Q, which were identified in 2/27 (7.4%) children in the homozygous p. 138Q/Q) or compound heterozygous ( p.138Q/229Q) with sporadic SR-FSGS.22 There are no reports of *NPHS2* causative mutations in African ancestry children with sporadic FSGS, probably reflecting fewer children enrolled in studies.22

Common genetic variants in the *APOL1* gene encoding apolipoprotein L1 are strongly associated with glomerular diseases, including hypertension-attributed chronic kidney disease and end stage renal disease (odds ratio (OR) 7), FSGS (OR 17), and HIV-associated collapsing glomerulopathy (OR 29) in African Americans. 23,24 *APOL1* genetic variants are frequent in West Africa and in the West African diaspora populations, including African Americans, but are absent on non-African chromosomes.25 In African Americans, approximately 70% of primary FSGS is attributed to carriage of two *APOL1* renal risk alleles, with peak age of onset between 15-39 years, although children with *APOL1*-associated FSGS have been identified with onset between 2-9 years old. 24,26 The *APOL1* genotype does not influence steroid responsiveness in FSGS or proteinuria response to cyclosporine or mycophenolate mofetil/dexamethasone in children and young adults with SRNS.26 In South Africa, *APOL1* high risk genotype is strongly associated with HIVAN in Black adults (OR 89), but its role in FSGS in Black African adults is unresolved and has not been investigated in children.27

The genetic basis for the racial disparity in SRNS and FSGS histology of children with NS from sub-Saharan Africa is unknown, and no genetic studies have been reported to date. We hypothesized that a genetic mutation or variant more frequent in Black children than in Indian or White South African children is responsible for their higher rates of SRNS. Seeking the genetic basis of SRNS, we performed mutational analysis of the *NPHS2* gene and determined *APOL1* genotypes in DNA from Indian and Black children with SSNS and SRNS and healthy donor controls. We identified a shared *NPHS2* autosomal recessive mutation in 30% of Black children with SR-FSGS. Our results were replicated in a second cohort of SR-FSGS Black cases and age and race matched controls.

**Materials and Methods**

**Subjects**

Unrelated children with sporadic SRNS (n=60) or SSNS (n=19) were enrolled at the King Edward VIII Hospital and Inkosi Albert Luthuli Central Hospital, Durban, South Africa during the period January 2005 to December 2011. Both hospitals are tertiary referral centers for children with complex kidney diseases for the Province of KwaZulu-Natal and its neighboring provinces. All children with NS were given enalapril, an ACE inhibitor and oral prednisone (2mg/kg, maximum 60 mg) for 6 weeks followed by the same dose on alternative days for another 6 weeks and reduced to none over 2.5 months. Failure to respond was taken as steroid resistance in accordance with standard criteria Barratt M.28 Second line treatment included enalapril and low dose oral prednisone given on alternate days with a daily dose of oral cylophosphamide for 8 to 12 weeks. Children with SRNS who did not respond to oral cyclophosphamide plus prednisone received intensive treatment with intravenous methyprednisolone (n=11) or intravenous cyclophosphamide (n=7) or both MP plus IV cyclophosphamide (n=2) or tacrolimus (n=14) or both IV cyclophosphamide plus tacrolimus (n=2) together with low-dose prednisone on alternative days. All kidney biopsies were interpreted using light microscopy, immunofluorescence and electron microscopy. Children with primary SSNS or SRNS and with a glomerular filtration rate (GFR) of >60ml/m2/min, corrected for body surface area, were eligible for entry into the study. The following tests were performed to exclude secondary forms of NS: antistreptolysin O titer (ASOT), hepatitis B and C screen, liver function tests, blood culture, Widal tests, Wasserman reaction, antinuclear factor, Epstein Barr virus, HIV testing, and cytomegalovirus testing. Proteinuric remission was defined as a protein to creatinine ratio <0.2 mg/ml and partial remission as a protein to creatinine ratio<1.9 mg/ml.

A group of 61 Black and 53 Indian healthy blood donors were enrolled as control groups.

For replication of *NPHS2* and *APOL1* genetic associations, we enrolled 20 cases with sporadic SRNS and biopsy-proven primary FSGS and 19 age-matched Black controls with no serological evidence of HIV infection and with normal kidney function and no proteinuria.

Written informed consent was obtained from the parents of children and children over 7 years assented; the Biomedical Research Ethics Committee of the University of KwaZulu-Natal, SA and the National Cancer Institute, NIH, USA approved this study.

**Sequencing and genotyping**

Genomic DNA was extracted using the PuregeneGenomic DNA Purification System, by Gentra Systems, Minneapolis, Minnesota, USA. DNA was quantified using the Nanodrop® ND-1000 spectrophotometer. Exons 1 through 8 were sequenced using ABI Prism BigDye Terminator on the ABI 3700 Analyzer at standard conditions. Primer sequences were from y et al.;18 for exon one, a new primer was designed within genomic regions flanking NPHS2 exon 1 (TGTAAAACGACGGCCAGTCGACTCCACAGGGACTGC  -NPHS2\_ex1\_Forward) and (CAGGAAACAGCTATGACC CCTTAGTTACCACCTGGA - NPHS2\_ex1\_Reverse). M13F (TGTAAAACGACGGCCAGT) and M13R (CAGGAAACAGCTATGACC) sequence tags were added to the primers to aid in sequencing.  Fragments were amplified using a TaKaRa LA Taq kit (TaKaRa, CloneTech), using GC Buffer I according to the manufacturer’s suggestion. PCR conditions were the following: 94 C for 1 minute followed by 30 cycles of 94 C for 1 minute; 57 C for 2 minutes 30 sec, followed by an extension at 72o C for 10 minutes. APOL1 G1 and G2 risk alleles were genotyped using TaqMan assays as previously described.24 For a coalescence analysis to determine the age of 74 Black controls and 10 p.V260E homozygotes using the Human Exome Chip (HumanExome-12-v1-2-B) for determination of haplotype length similarity and to determine the age of the variant in the South African Black population.

**Statistical Methods**

Fisher’s exact test was used for categorical tests. T-tests were used to compare the age distributions between Indian and Black SRNS, and between Black SRNS with and without V260E homozygosity; p values for the Mann-Whitney test were similar. A binomial confidence interval was used to estimate the frequency of *NPHS2 V260E* in the normal population. All statistical tests and simulations were done in R.

We determined relatedness among *NPHS2* V260E homozygotes by estimating the age of the most recent common ancestor for the V260E mutation among our subjects using coalescence29,30. We examined homozygosity of Chr. 1 SNPs typed on the Illumina Exome chip V2.1, by plotting heterozygous and homozygous SNPs for each subject in the region around NPHS2 (Supp. Fig. 1). Subjects inheriting the mutation from a recent common ancestor will have inherited identical segments of chromosome around the mutation from their two parents, with length determined by recombination since the common ancestor. The expected distribution of lengths of homozygosity was calculated by a simulation of recombination over the of number of generations since the common ancestor, and compared with observed distribution.

**Results**

Seventy-nine unrelated children with sporadic NS were enrolled into the study between 2005 and 2011(Table 1). Mothers of the affected children reported no affected siblings or familial history of kidney disease. Steroid resistance was significantly more frequent in Black (97.4%) compared to Indian (55%) children with NS (p=8 x 10-6). Indian children with SRNS were more likely to be male than Black children (72% vs 45%, p =0.06). The mean age of nephrotic syndrome presentation was 91.7 months for Indian children and 85.6 months for Black children (p=0.6). FSGS was the most common histopathology in both Black (81.6%) and Indian (72%) children with SRNS. Children responding to oral steroids (steroid sensitive) were not biopsied.

As previous studies identified *NPHS2* autosomal recessive mutations as a major cause of familial and sporadic SRNS in children presenting after 3 months of age, we sequenced *NPHS2* exons in steroid sensitive and steroid resistant cases and controls. Supplementary Table 1 lists the *NPHS2* variants identified in Indian and Black cases and controls for which we had unambiguous sequencing data; the percentage is based on the number of subjects with interpretable sequence at the variant site. We observed seven missense variants: p.P20L, p.G42R, p.A61V, p.R229Q, p.A242V, p.V260E, all of which have been previously reported, and a novel variant, p. P369S, which has not been reported in 1000 genomes or other public databases and is predicted to be benign (Supplementary Table 1). *NPHS2* p.V260E is a known pathogenic mutation in the homozygous state and *NPHS2* p. R229Q is a known pathogenic mutation, but only causes SR-FSGS compound heterozygosity with certain other *NPHS2* pathogenic mutations in trans configuration.31 With the exception of *NPHS2* V260E, no missense variants were observed in the homozygous or compound heterozygous state in any Black or Indian steroid sensitive or steroid resistant cases. *NPHS2* mutations in the heterozygous state are not predicted to cause disease; weighted and unweighted gene burden tests for these variants did not reveal an excess of variants in nephrotic syndrome cases versus controls (p>0.5).

Notably, the *NPHS2* V260E mutation was present in the homozygous state in 8 of 30 (26.7%) Black SRNS cases with unambiguous sequencing results; all p.260E homozygotes had biopsy-confirmed FSGS (Table 2). Among Black children with FSGS, homozygosity for p.260E accounted for 33% (8/24) of FSGS (p=3x10-7). The p.V260E mutation was observed in the heterozygous state in one Black control. *NPHS2* p.V260E was not observed in Black or Indian steroid sensitive nephrotic syndrome patients, Black steroid resistant patients with other histologies or in any Indian cases controls (Table 2).

To replicate the p.260E/E association with SR-FSGS, we sequenced DNA from a second cohort of 20 Black children with sporadic SR-FSGS and 19 race-and age-matched controls. Of the children with SR-FSGS, 6/20 (30.0 %) were homozygous for the p.V260E mutation whereas none of the controls carried the variant. Taken together, the p.260E/E variant accounted for 14/44 (31.8%) children with sporadic SR-FSGS. Black SR-FSGS cases homozygous for p.V260E had an earlier age of onset compared to steroid resistant nephrotic cases not carrying the p.260 E/E genotype (mean age 68 months versus 98 months, respectively, P=0.025) (Figure 1). The deleterious mutation was found in the heterozygote state in 1/ 73 controls sequenced, for an observed population allele frequency of 0.7%, (95% CI: 0.02%, 4%) (Table 3).

As *APOL1* G1 and G2 risk alleles are strongly associated with sporadic FSGS in African Americans, we genotyped *APOL1* G1 and G2 risk variants in the combined case and control groups; only 3 individuals with two APOL1 risk alleles were observed, all of whom had SR-FSGS (Table 3). Two of the three SR-FSGS cases were also homozygous for the p.V260E mutation. Dual homozygosity for *APOL1* and p. V260E was not predictive of age of nephrotic syndrome onset: the child carrying two copies of the deletion variant (G2/G2) was diagnosed with NS at 28 months and the second child carrying two copies of the missense variant (G1/G1) was diagnosed at 146 months. The child with SR-FSGS carrying only the *APOL1* G2/G2 high-risk genotype was diagnosed at 46 months. The minor allele frequencies for the G1 and G2 alleles were 9.0% and 10.1%, respectively, in controls compared to 12.2% and 15.1%, respectively, in cases.

**Response to therapy**

We measured response to therapy by protein-creatinine ratio and serum albumin level. Complete remission was defined as protein-creatinine ration < 0.2 and serum albumim > 30g/dL. Partial remission was defined as not meeting the criteria for full remission, with protein-creatinine ratio < 1.9 and and serum albumim > 25g/dL. No subjects carrying p. 260 EE (N = 14) had either complete or partial remission in response to therapy (table 4), while 9 subjects carrying p. 260 VV (N = 32) had complete remission, and 6 partial remission. Six children carrying p 260E/E were further treated with tacrolimus (n=3) or intravenous cyclophosomine (n=3), but none showed proteinuric response. Combining complete and partial remission, the difference in treatment response between p. 260 EE and VV was highly significant, p = 0.002.

**Founder effect and age of variant**

The established association of *NPHS2* p.V260E with consanguineous familial SR-FSGS suggested that our subjects might have a cryptic consanguinity by descent from a recent common ancestor. This would imply that, for both copies of chromosome 1 in these individuals, the region surrounding *NPHS2* would be identical by descent, creating an extended region of homozygosity around the variant. The average length of homozygosity is a function of the number of generations since the common ancestor.

Sufficient DNA remained for 30 cases and 54 controls, including 10 individuals carrying two copies of *NPHS2* p.V260E, to genotype using the Illumina exome chip. This chip provides good coverage of the region of interest, with 1674 markers within 20 megabases (Mb) of *NPHS2*. Two of the ten p.V260E homozygotes had segments of homozygosity around *NPHS2* of 13 and 14 Mb, while the eight remaining had segments ranging from 1.9 to 3.6 Mb.

We compared the length of the shorter segments with output of a simulation of recombination around the given locus, for different numbers of generations. While the longer stretches of homozygosity are consistent with fairly few generations, the size and number of shorter segments indicated a 95% confidence interval of for the number of generations since the last common ancestor of 25 to > 48 generations. These results suggest that the mutation appeared in this population between 500 and 1000 years ago.

Coefficient of relatedness tests using genome-wide data from exome chip indicate that parents of two of the children carrying NPHS2 p.V260E were 2nd cousins; the other 8 of the children did not show cryptic relatedness.

**Discussion**

We have shown that a shared pathogenic mutation, *NPHS2* p.V260E, accounted for 23% of steroid resistant nephrotic syndrome and 30% of SR-FSGS among Black children in KwaZulu-Natal Province, South Africa presenting with NS. This was an unexpected result since published data suggest that a single-gene cause of sporadic SR-FSGS is identified in <10% of children. Children homozygous for *NPHS2* V260E developed disease at a younger age compared to non-carriers, consistent with the severity of disease previously associated with *NPHS2* V260E.15 We also found that *APOL1* renal risk variants, which are strongly associated with FSGS in children and adults with West African ancestry in the USA, were not significant contributors to nephrotic syndrome or SR-FSGS in southern African Black or Indian children with nephrotic syndrome.24,26 This may be due to the lower frequency of *APOL1* risk alleles in the population, and /or the reduced prevalence of other genetic and/or environmental factors that interact with *APOL1* risk alleles to induce glomerular injury and/or to the younger age of our patient population.26

Calcineurin inhibitors, especially cyclosporine, are frequently used to treat SRNS and is recommended for improving outcomes by Kidney Disease Improving Global Outcomes (KDIGO). Tacrolimus has also achieved excellent remission rates in children with FSGS with remission rates of 85.7%, with fewer side effects than cyclosporine. There have been few systematic studies of CSA or Tacrolimus on children with genetic FSGS due to autosomal recessive mutations in podocin structural genes with alter the podocyte ultrastructure. One study showed no response to cyclosporine in 2 children with SRNS and a partial response in a third child, all of whom where homozygous for p.R138Q,32 a mutation which shares pathogenic features with p.V260E; both mutations encode proteins that are trapped in the endoplasmic reticulum (ER) and are unavailable for nephrin binding. Other studies indicate that children with NPHS2 mutations do not benefit from immunosuppressive agents and/or angiotensin-converting enzyme inhibitors.33

The p.V260E mutation appears to be a rare, but not extremely rare, variant in this southern African population, as we observed 1 heterozygote in a healthy Black blood donor. To elucidate the population history of this mutation and to determine if consanguinity or cryptic relatedness accounted for the high rate of p. E260V in Black Africans with NS, we genotyped 10 subjects homozygous for p.V260E and 64 SRNS cases and controls without the mutation with the Illumina Exome chip. Sharing of p.V260E due to consanguinity would be revealed by a region of extended homozygosity around the mutation. Overall, parents of children carrying p. V260E showed no sign of recent consanguinity, but rather showed evidence, in the length of homozygous segments around NPHS2, of descent from a common ancestor 20-48 generations removed from the present, indicating that the high frequency of this variant in SR-FSGS is not due to a recent common ancestor, and suggesting a rare but widespread presence on chromosomes among the Black population.

Previous observations of SRNS caused by *NPHS2* V260E have all been in Omani families or in families in Bahrain or the Comoros Islands, previously part of the Omani empire of the late 17 to 19th centuries, prompting the hypothesis that the mutation may have spread with this empire.34 The Omani empire expanded into the Swahili (East African) Coast in 1690s, where ancestors of the Durban Zulu population lived; however the age of the mutation in the studied population suggests an appearance prior to this time and makes it unlikely that it was introduced by Omani influence. It remains to be shown that *NPHS2* p. V260E in our population is related to previously observed p.V260E, rather than being independent mutations. The relationship could be confirmed by identity of haplotypes surrounding *NPHS2* p.V260E in the different populations.

Our study is limited in that the number of individuals is small, and the results for the studied population, Black Africans of the KwaZulu-Natal province, may not be generalizable to other Black ethnic groups. However the strength of the association in our study, and the indicated age of the mutation, suggest that the mutation and its profound effects may be present in a broader population.

The identification of p.V260E will inform the differential diagnosis, prognosis, and treatment in Black children presenting with NS. Incidence and prevalence rates of SSNS and SRNS in Black and Indian children are not systemically collected, but hospital records at a referral center suggest that 60% of Indian children and 90% of Black children are steroid resistant.35 In Durban, South Africa, all children presenting with NS are treated with steroids before kidney biopsy; however, Indian children with relapsing NS and steroid dependent NS are given a trial of oral cyclophosphamide before kidney biopsy whereas Black children with SRNS are given a kidney biopsy immediately, as most do not respond to cyclophosphamide.6 Genetic testing of black children NPHS2 p.V240E homozygosity would provide a precise genetic diagnosis of SR-FSGS in homozygous carriers, thereby sparing a substantial subset of children an unnecessary kidney biopsy (Figure 2). Clinical trials will be necessary to determine optimal treatment for homozygous carriers of *NPHS2* p.V260E and particularly responsiveness to treatment with calcineurin inhibitors (cyclosporine and tacrolimus) and other extensive treatment modalities.

In summary, the high frequency of *NPHS2* p.V240E homozygosity among unrelated children with sporadic SRNS/FSGS makes testing for NPHS2 p.V260E in Black African children presenting with NS a valuable application of precision medicine, reducing stress on patients and expense on overburdened health care systems. Identification of this variant should be a part of differential diagnosis, is a cost-effective alternative to kidney biopsy in homozygous carriers, and identifies with precision a large subset of patients who are unresponsive to immunosuppressive agents (i.e., oral steroid treatment and cyclophosphamide), sparing these children the potential severe and life threatening adverse effects of these agents. The applicability of this approach may be much larger, as the variant is likely to be present in a much larger population; further studies are vital to define the extent of *NPHS2* p. V260E in children with SRNS in related black African populations.

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References

**Figure Legends**

Figure 1. Comparison of distributions of ages of onset of steroid resistant individuals between carriers of two copies of the mutant allele (N = 14) and carriers of two copies of the wild-type allele (N = 37) at *NPHS2 V260E*. There were no heterozygotes for this locus in the SRNS group. Data are from the combined discovery and the replication cohorts.

Figure 2. Targeted genetic approach for Black African children with nephrotic syndrome.

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Figure 1.

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Figure 2.

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| **Table 1. Demographic and clinical characteristics of children with nephrotic syndrome in the discovery and replication cohorts.** | | | | | | |
|  | **Discovery** | | | | **Replication** | |
| **Disease entities** | **Black nephrotic syndrome cases**  **N=39** | **Indian nephrotic syndrome cases**  **N= 40** | | **OR, (95% CI),**  **p value** | **Black SR-FSGS Cases**  **N=20** | |
| **Steroid sensitive nephrotic syndrome** | 1 (3%) | 18 (45%) | | OR 30 (4, 1320)  P=8x10-6 | 0 | |
| **Steroid resistant nephrotic syndrome** | 38 (98%) | 22 (55%) | | 20 | |
| Age range (months) | 1-168 | 37-169 | | p = 0.6 | 26-165 | |
| Mean age (months)  [Median probably would be more appropriate, assuming non-normal distribution] | 85.6 | 91.7 | | 101 | |
| Males | 17 (45%) | 16 (72%) | | 3.2 (0.9, 12.4)  p=0.06 | 13 (62%) | |
| Females | 21 (55%) | 6 (28%) | | 8 (38%) | |
| **Histology of SRNS** |  |  | |  |  | |
| Focal segmental glomerulosclerosis | 31 (82)% | 16 (73%) | | 1.6 (0.4-6.9)  p=0.5 | 20 (100%) | |
| Interstitial nephritis | 2 (5%) | 1 (4%) | | ND | - | |
| Mesangioproliferative glomerulonephritis | 3 (8%) | 1 (4%) | | ND | - | |
| Mesangial sclerosis | 1 (3%) | 0 | | ND | - | |
| Proliferative glomerulonephritis | 1 (3%) | 0 | | ND | - | |
| Glomerulonephritis | 0 | 2 (9%) | | ND | - | |
| Minimal change disease | 0 | 2 (9%) | | ND | - |
| Shown are the numbers (percent) of steroid sensitive and steroid resistant nephrotic syndrome (SRNS). Black children were much more likely than Indian descent children to have steroid resistant nephrotic syndrome (odds ratio 30). | | | | | |

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| **Table 2. NPHS2 p.V260E in Black and Indian SSNS and SRNS with nephrotic syndrome in the discovery cohort.** | | | | |
|  | **Black nephrotic**  **syndrome cases** | | **Indian nephrotic syndrome cases** | |
|  | p.260V/V | p.260E/E | p.260V/V | p.260E/E |
| Total | 23 | 8 | 40 |  |
| Steroid sensitive NS | 1 | 0 | 18 | 0 |
| Steroid resistant NS | 22 | 8 | 22 | 0 |
| FSGS | 16 (67%) | 8 (33%) | 16 (100) | 0 |
| Other histologies | 6 (100) | 0 | 6 (100) | 0 |
| Shown are the numbers (frequency) of the NPHS2 p. V260E variant in the nephrotic syndrome discovery cohort, including those who were steroid sensitive (SSNS) and steroid resistant (SRNS). Unambiguous DNA sequence data for V260E was available for 31/38 Black SRNS children and 22/22  SRNS Indian children. | | | | |

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| **Table 3. Association of NPHS2 p. 260 E/E in Black children with biopsy-proven steroid resistant FSGS from the discovery and replication cohorts.** | | | |
| ***NPHS2* genotype** | **Steroid resistant FSGS**  **N=44** | **Controls**  **N=73** | **OR (95%CI), FET** |
| **Discovery cohort** | | | |
| p. 260 V/V | 16 (67%) | 54 (98%) | Reference |
| p. 260 E/V | 0 | 1 (2%) | Not significant |
| p. 260 E/E | 8 (33%) | 0 | Infinite (4.9, infinite)  3 x 10-5 |
| **Replication cohort** | | | |
| p. 260 V/V | 14 (70%) | 18 (100%) | Reference |
| p. 260 V/E | 0 | 0 | -- |
| p. 260 E/E | 6 (30%) | 0 | Infinite (1.2, Infinite)  0.02 |
| **Combined cohorts** | | | |
| p. 260 V/V | 30 (68%) | 72 (99%) | Reference |
| p. 260 V/E | 0 | 1 (1%) | Not significant |
| p. 260 E/E | 14 (32%) | 0 | Infinite (7.2, Infinite)  3 x 10-7 |
| FET, Fisher exact test; this table restricted to only individuals with SR-FSGS and controls. Unambiguous *NPHS2* sequence results were available for 44/51 FSGS cases and 73/79 controls. | | | |

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| **Table 4. *APOL1* association with SR-FSGS among Black children from the discovery and replication cohorts.** | | | | | | |
| ***APOL1* Genotype\*** | **All steroid resistant-FSGS** | | | **Restricted to steroid-resistant FSGS without *NPHS2* homozygosity** | | |
|  | Cases  41 | Controls  79 | OR  (95% CI) | Cases  30 | Controls  79 | OR  (95% CI) |
| Low risk genotype | 39 (93%) | 77  (98%) | Reference | 29  (97%) | 77 (98%) | Reference |
| High risk: genotype | 2 (7%) | 2 (2%) | 2.9  (0.32-36.5) | 1  (3%) | 2 (2.5%) | 1.3  (0.02-26.3) |
| G1 + G2 allele frequency | 27% | 19% | 1.6  (0.82-3.1) | 23% | 19% | 1.3  (0.58-2.8) |
| The high risk APOL1 genotype, comprising two copies of the risk allele (G1/G1, G2/G2 or G1/G2), was not associated with FSGS among Black children. *APOL1* genotypes were available for 41/51 FSGS cases and 79/80 controls. APOL1 risk variants were not observed in Indian children. | | | | | | |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Supplementary Table 1. NPHS variants detected in Indian and black African children with nephrotic syndrome and controls. | | | | | | | | | | | | | | |  |  |
| **Variant** | **Allele** | **All**  **SSNS**  **No=19** | | **Indian SRNS**  **No =19** | | **Indian**  **Controls**  **No =49** | | **Black**  **SRNS**  **No =35** | | **Black**  **Controls**  **No=55** | | **1000GP**  **or dbSNP** | **SIFT prediction** | **PolyPhen prediction** | **Reported association**  **with FSGS** | **Ref** |
|  |  | **Het** | **Hom** | **Het** | **Hom** | **Het** | **Hom** | **Het** | **Hom** | **Het** | **Hom** |  |  |  |  |  |
| -52 5’UTR | G/T | 0 | 0 | 0 | 0 | 0 | 0 | 4  15% | 0 | 14  31% | 5  11% | rs78541594 |  |  | Uncertain, ESRD progression | [McKenzie et al., 2007] |
| -51 5’UTR | G/T | 2  20% | 0 | 6  35% | 0 | 10  24% | 0 | 5  19% | 8  36% | 12  22% | 0 | rs12406197 |  |  | No | Yu et al., 2005] |
| p. P20L | c.59C>T | 1  7% | 0 | 1  6% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | rs74315344 | Deleterious | Possibly damaging | Yes, pathogenic for SRNS | [Boute et al., 2000; Ruf et al., 2004] |
| p. G34G | c.102G>A | 5  36% | 0 | 4  22% | 0 | 9  20% | 0 | 7  25% | 1  4% | 16  29% | 3  5% | rs1079292 |  |  | No | [Karle et al., 2002] |
| p. G42R | G/A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4  7% | 1  2% | rs559836164 | Tolerated | Benign | No |  |
| p. S48S | C/T | 0 | 0 | 0 | 0 | 1  2% | 0 | 2  7% | 0 | 5  9% | 0 | rs111306764 |  |  | No |  |
| p. A61V | c.182C>T | 0 | 0 | 0 | 0 | 1  2% | 0 | 1  3% | 0 | 0 | 0 | rs201050491 | Tolerated | Benign | Yes | [Dusel et al., 2005] |
| p. R229Q  4% | c.686G>A | 0 | 0 | 0 | 0 | 1  2% | 0 | 1  (3%) | 0 | 0 | 0 | rs61747728 | Tolerated | Possibly damaging | Yes, deleterious as  Compound het with  pathogenic I  mutation | [Tsukaguchi et al., 2002] |
| p. A242V | c.725C>T | 1  7% | 0 | 0 | 0 | 1  2% | 0 | 4  13% | 0 | 4  8% | 0 | rs61747727 | Deleterious | Probably damaging | Yes, pathogenic for  SRNS | [Weber et al., 2004] |
| **p. V260E** | T/A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **8**  **27%** | **1**  **2%** | 0 | CM042098 reported in HGMD | Damaging | Probably damaging | Yes, pathogenic | Weber et al., 2004 |
| p. A297A | c.891G>A | 0 | 0 | 0 | 0 | 1  2% | 0 | 1  3% | 0 | 0 | 0 | rs5005771 |  |  | No | [Dusel et al., 2005] |
| p. A318A | c.954T>C | 8  61% | 1  8% | 11  55% | 4  20% | 27  54% | 16  32% | 12  38% | 5  9% | 32  56% | 15  26% | rs1410592 |  |  | No | [Karle et al., 2002] |
| p. L346L | c.1038A>G | 0 | 0 | 3  5% | 0 | 3  6% | 0 | 5  16% | 0 | 4  7% | 0 | rs3818587 |  |  | No | [Boute et al., 2000] |
| p. P369S | C/T | 0 | 0 | 0 | 0 | 1  2% | 0 | 0 | 0 | 0 | 0 | This study | Tolerated | Benign | Uncertain | This study |

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