

Brazilian Network of Pediatric Nephrotic Syndrome (REBRASNI)



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Pediatric nephrotic syndrome (PNS) is a major medical problem without a known cure nor clear treatment regimen and has been the focus of intense investigation. Its most severe manifestation is steroid-resistant nephrotic syndrome (SRNS), the second most frequent cause of pediatric end-stage kidney disease (ESKD).¹

New concepts about PNS have been built on fundamental genetic discoveries. Aided considerably by the advent of next-generation sequencing and its application to familial cases in the research setting, more than 60 single gene (“Mendelian”) causes of SRNS have been discovered. Non-Mendelian genetic forms of nephrotic syndrome (NS) also have been identified, including high-risk *APOL1* genotypes with focal and segmental glomerulosclerosis^{2,3} and *HLA-DQ* alleles with pediatric steroid-sensitive NS.⁴

The translation of scientific knowledge into clinical outcomes requires robust clinical trials and epidemiological studies with appropriate size, design, and comprehensiveness. Studies in the United States, the United Kingdom, and the European Union, such as NEPTUNE, PodoNet, and PredNos, are driving forward rigorous and novel scientific inquiries in PNS. However, there is a noted lack of similar studies being

performed outside of developed countries. It is already clear that the prevalence and natural history of NS differs by geographic location and genetic ancestry, with prevalence of Mendelian SRNS differing across countries, and *APOL1*-associated NS being a condition specific to those of recent African ancestry. This creates an opportunity for studies of NS in developing countries to add significant information to the field.

Brazil is the fifth largest country in the world, with a highly admixed population, derived mainly from European colonizers and immigrants, African slaves, and indigenous Amerindians. The diversity of people and climates provides an opportunity to study the genetic and environmental influence on NS, in a way that has not been matched elsewhere around the world. It is possible, moreover, that the prevalent tropical climate present in Brazil may influence the prevalence and diversity of infections, potentially affecting PNS epidemiology.

In the past 6 years, research on the genetics of PNS in Brazil has gained momentum in main universities of São Paulo. This interest has been translated into some reports (Watanabe A, Neves PD, Watanabe EH, et al. *APOL1* risk alleles are critical for the development of collapsing glomerulopathy in Brazilian children [abstract]. *J Am Soc Nephrol*. 2018;29:716).^{5–8}

THE REBRASNI

In light of the need to better understand the etiology and natural history of NS, and the unique opportunity to do this in Brazil, physicians and physician scientists from the Divisions of Pediatric Nephrology and Nephrology of 3 renowned Brazilian medical schools, University of São Paulo, Federal University of São Paulo, and State University of Campinas, created the Brazilian Network of Pediatric Nephrotic Syndrome (REBRASNI, *Rede Brasileira de Síndrome Nefrótica na Infância*, in Portuguese) early in 2018 (Figure 1). The mission of REBRASNI is to generate knowledge to improve diagnosis, evaluate prognosis, and contribute to personalized treatment and potential cure of PNS.

REBRASNI uses an electronic platform (www.rebrasni.sites.unifesp.br) to register cases of PNS within the Brazilian territory. Epidemiologic, clinical, and laboratory data will be prospectively obtained from all patients with NS, whereas biosamples will be initially collected from individuals with SRNS. Patient samples (whole blood and urine) and a fragment of kidney biopsy (when clinically indicated) will be stored in biorepositories, according to Brazilian regulations (see the section Research Plans

later in the article, [Supplementary Methods](#), and [Figure 2](#)).

RESULTS

Preliminary data have been collected in the state of São Paulo. There were 454 patients with NS within the age range of 0.1 to 18 years followed between 2017 and 2018 at State University of Campinas and University of São Paulo, and 1 major affiliated center: University of São Paulo at Ribeirão Preto. The median age of NS onset was 3.1 (0.3–14.9) years; 268 patients (57.3%) were male, and 349 (76.9%) were Caucasian and 95 (20.9%) were mixed race/black (Table 1). Eighty-seven of them (19.2%) harbored the clinical diagnosis of SRNS/congenital nephrotic syndrome. Of these 87 patients, 30 (34.5%; or 6.6% of all patients with NS) developed ESKD.

Seventy of the 87 patients with SRNS (80.4%) had undergone a kidney biopsy. Forty-four (62.9%) had the diagnosis of focal and segmental glomerulosclerosis and 8 (11.4%) collapsing glomerulopathy. Seven of the patients with collapsing glomerulopathy (87.5%) developed ESKD, a condition not reached by any patient with minimal change disease during the 2-year follow-up. In biopsied patients with steroid-



Figure 1. Structure and initiatives of Brazilian Network of Pediatric Nephrotic Syndrome (REBRASNI). UNICAMP; State University of Campinas; UNIFESP, Federal University of São Paulo; USP, University of São Paulo.

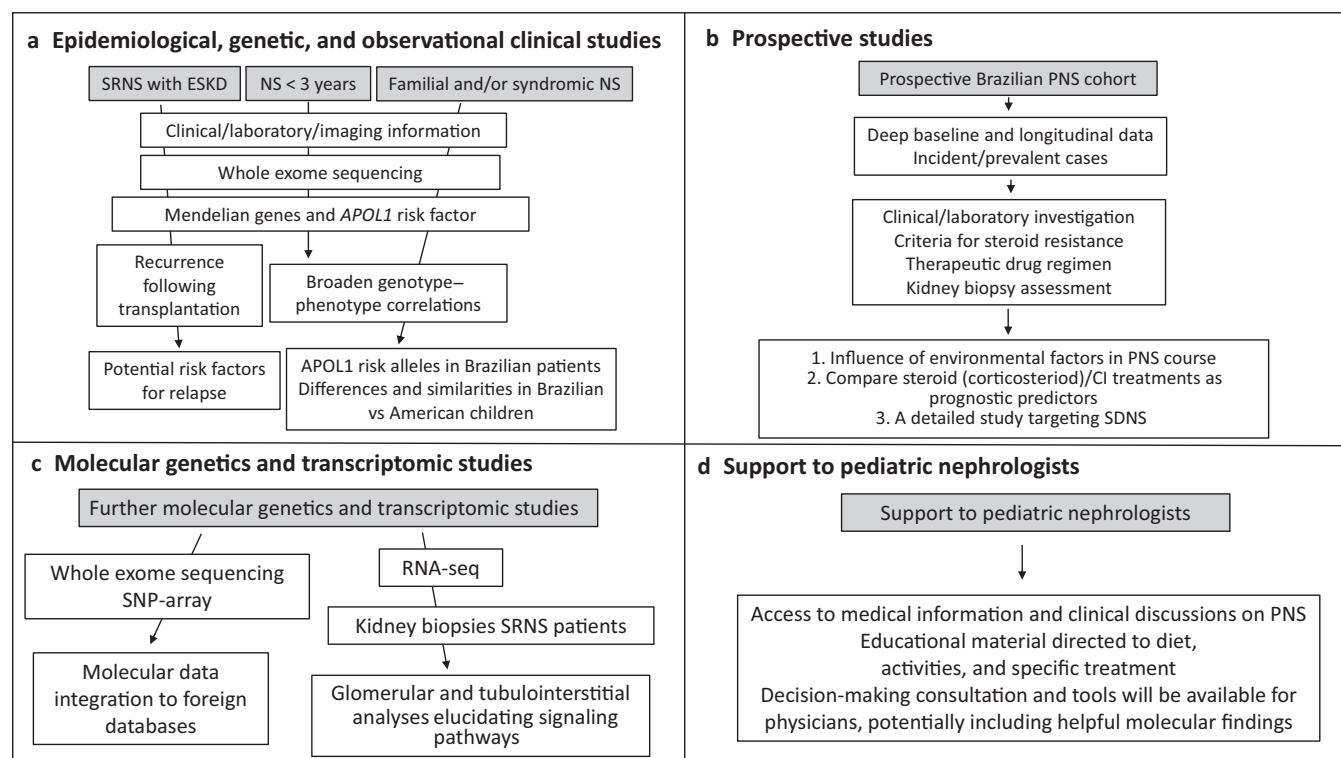


Figure 2. Research plans. CI, calcineurin inhibitor; ESKD, end-stage kidney disease; NS, nephrotic syndrome; PNS, pediatric nephrotic syndrome; RNA-seq, RNA sequencing; SDNS, steroid-dependent nephrotic syndrome; SNP, single nucleotide polymorphism; SRNS, steroid-resistant nephrotic syndrome.

dependent NS, the most common diagnosis was minimal change disease (Table 2).

Among the 1606 children with ESKD submitted to kidney transplantation at Federal University of São Paulo, University of São Paulo-São Paulo, and State University of Campinas (1982–2018), 154 (9.6%) had the primary diagnosis of NS. A total of 135 patients have available medical records for appropriate studies. Eighty-three of them (61.5%) were male, the median age of NS onset was 4.0 (0.2–15.0) years, the median time to ESKD was 4.0 (1.0–15.2) years, and the median age of first kidney transplantation was 12.0 (2.5–18.7) years. Thirty-one patients (23.0%) had NS recurrence after transplantation (Table 3).

The high proportion of SRNS, ESKD, and steroid dependency observed in our cohort is likely explained

by the fact that State University of Campinas, University of São Paulo, and Federal University of São Paulo are tertiary pediatric nephrology units to which a significant number of patients with difficult-to-treat NS and ESKD cases are referred.

RESEARCH PLANS

One of the most important aims of REBRASNI is to discover the genetic architecture and describe the epidemiologic characteristics of PNS in Brazil. To accomplish these goals, our network will prioritize performance of a number of studies in the settings

Table 2. Histological diagnoses of biopsied pediatric patients with nephrotic syndrome followed at State University of Campinas, University of São Paulo-São Paulo, and University of São Paulo-Ribeirão Preto

Variable	Steroid-resistant nephrotic syndrome	Steroid-dependent nephrotic syndrome
Number of biopsied patients, n/N (%)	70/87 (80.4)	67/147 (45.6)
Minimal change disease, %	18.6	56.7
Focal and segmental glomerulosclerosis, %	62.9	34.3
Collapsing glomerulopathy, %	11.4	0.0
Diffuse mesangial sclerosis, %	4.3	0.0
Acute tubular necrosis, %	0.0	3.0
Tubulointerstitial nephropathy, %	0.0	1.5
Proliferative mesangial glomerulopathy, %	2.9	1.5
Focal interstitial fibrosis, %	0.0	1.5
Membranous nephropathy, %	0.0	1.5

Table 1. Demographics and age of onset of patients with pediatric nephrotic syndrome followed at State University of Campinas, University of São Paulo-São Paulo, and University of São Paulo-Ribeirão Preto

Variable	Result
Total number of patients	454
Sex, %	Male: 57.3 Female: 42.7
Median age at onset (range) of nephrotic syndrome, yr	3.1 (0.1–14.9)
Ethnicity, %	Caucasian: 76.9 Mixed/Black: 20.9 Other: 2.2

Table 3. Demographics and clinical features of kidney transplantation in patients with pediatric nephrotic syndrome followed at Federal University of São Paulo, University of São Paulo–São Paulo, and State University of Campinas

Demographic and clinical features	Result
Time period	1982–2018
Number of transplanted patients with nephrotic syndrome with available data	135
Sex, %	Male: 61.5 Female: 38.5
Median age at onset (range) of nephrotic syndrome, yr	4.0 (0.2–15.0)
Median time to end-stage of kidney disease (range), yr	4.0 (1.0–15.2)
Median age at first kidney transplantation (range), yr	12.0 (2.5–18.7)
Number of patients with recurrence of nephrotic syndrome in the graft (first kidney transplantation), <i>n/N</i> (%)	31/135 (23.0)

itemized as follows, summarized in Figure 2, and more comprehensively described in the [Supplementary Methods](#).

Epidemiologic, Genetic, and Observational Clinical Studies

- (i) Genotype–phenotype correlations based on whole exome sequencing (including *APOL1* genotyping) and a broad spectrum of clinical, biopsy, image-nologic, and laboratory characterization, will address the following outcomes:
 - ESKD;
 - onset when younger than 3 years old; and
 - familial and/or syndromic NS.
- (ii) Clinical analyses will aim to identify and characterize potential risk factors for occurrence of NS relapse following kidney transplantation, treatment efficacy, and safety.

Prospective Studies

- (i) Establish a prospective Brazilian PNS cohort:
 - to evaluate the influence of environmental factors associated with the onset and/or clinical course of PNS;
 - to compare failure of steroid and calcineurin inhibitor treatments as prognostic predictors of progression of chronic kidney disease in PNS;
 - to study the potential roles of persistent microscopic hematuria, selective proteinuria index, level of hypoalbuminemia, and/or new biomarkers in the PNS clinical course; and
 - to study steroid-dependent patients with a particular focus on steroid pharmacokinetics.

Molecular Genetics and Transcriptomic Studies

- (i) Children without a known Mendelian cause of their disease will undergo expanded, exome-wide genetic analysis. Molecular genetics data will be integrated into worldwide, NS databases and are

also expected to provide independent families to strengthen Mendelian claims of new SRNS-associated genes.

- (ii) Analyze the clinical impact of *APOL1* risk alleles in Brazilian patients with SRNS and factors that modify its penetrance.
- (iii) Study glomerular and tubulointerstitial transcriptomic analyses in kidney biopsies of SRNS REBRASNI patients using RNA sequencing.

Support to Pediatric Nephrologists and Families

- (i) Create access to medical information in PNS through scientific papers and clinical discussions via online site and provide educational material to patients focused on diet, activities, and specific treatments.

Financial Support and Sustainability

Funding from grant mechanisms, including binational ones, are expected to be the main source of financial support along the next years, followed by private donations. Applications to government funds and private health institutions are under way.

CONCLUSION

Brazilian pediatric nephrology is hoping and expecting that the success of REBRASNI may bring a major step forward in PNS knowledge and actions in our country and worldwide.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

[Supplementary File \(Word\)](#)

Supplementary Methods.

Figure S1. REBRASNI—Initial patient form.

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Identification of Two Forms of Human Plasma Renalase, and Their Association With All-Cause Mortality



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Renalase (RNLS) is a recently discovered flavoprotein produced and secreted by a variety of tissues including the kidneys.^{1–3} Inside the cell, RNLS as a nicotinamide adenine dinucleotide (NADH) oxidase regulates energy metabolism.⁴ Outside the cell, RNLS acts as a potent pro-survival signal when it binds to its cell membrane receptor, the plasma membrane calcium adenosine triphosphatase isoform PMCA4, and activates a variety of intracellular signaling pathways including the protein kinase B (AKT), extracellular-signal-regulated kinase (ERK), and signal transducer and activator of transcription 3 (STAT3) pathways.^{5–7} Administration of RNLS minimizes injury in *in vivo* models of myocardial infarction,⁸ ischemic tubular necrosis,⁹ and acute pancreatitis.^{S1} Conversely, RNLS deficiency in RNLS knockout mice exacerbates cisplatin-mediated acute and chronic renal injury, which is reversed by administration of RNLS.^{1,9,S2}

Dysregulated RNLS signaling appears to promote survival of malignant cells from several tumor types by augmenting expression of growth-related genes. Increased tissue RNLS expression in patients with pancreatic cancer and melanoma was associated with increased mortality.^{S3,S4}

No standardized and validated method of measuring RNLS concentrations in human plasma currently exists. In cohorts of subjects with normal renal function, a commercially available enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody yields widely variable results, with the concentrations ranging from as low as 1.18 ± 0.44 ug/ml (mean $\pm$ SD) to as high as 39.80 ± 14.63 (mean $\pm$ SD), a 33-fold difference.^{S5–S10}

We hypothesized that the variations in plasma concentrations measured by Western blot and commercially available ELISA may be due to the