Design of the Nephrotic Syndrome Study Network (NEPTUNE) to evaluate primary glomerular nephropathy by a multi-disciplinary approach

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Abstract

The Nephrotic Syndrome Study Network (NEPTUNE) is a North American multi-center collaborative consortium established to develop a translational research infrastructure for Nephrotic Syndrome. This includes a longitudinal observational cohort study, a pilot and ancillary studies program, a training program, and a patient contact registry. NEPTUNE will enroll 450 adults and children with minimal change disease, focal segmental glomerulosclerosis and membranous nephropathy for detailed clinical, histopathologic, and molecular phenotyping at the time of clinically-indicated renal biopsy. Initial visits will include an extensive clinical history, physical examination, collection of urine, blood and renal tissue samples, and assessments of quality of life and patient-reported outcomes. Follow-up history, physical measures, urine and blood samples, and questionnaires will be obtained every 4 months in the first year and bi-annually, thereafter. Molecular profiles and gene expression data will be linked to phenotypic, genetic, and digitalized histologic data for comprehensive analyses using systems biology approaches. Analytical strategies were designed to transform descriptive information to mechanistic disease classification for Nephrotic Syndrome and to identify clinical, histological, and genomic disease predictors. Thus, understanding the complexity of the disease pathogenesis will guide further investigation for targeted therapeutic strategies.

Introduction

Primary non-inflammatory glomerular diseases that include minimal change disease (MCD), focal and segmental glomerulosclerosis (FSGS) and membranous nephropathy (MN) are rare diseases that cause serious morbidity and high mortality accounting for approximately 15% of prevalent ESRD cases (2008) at an annual cost in the USA of more than $3 billion.[1, 2] Our limited knowledge of underlying glomerular disease mechanisms is a major barrier to successful therapeutic intervention. To date, this void has not been overcome by surrogate clinical and histopathology-based classification methods, which are of limited value in reliably predicting disease natural history or responsiveness to therapy. Hence, there is a need for basic and translational investigation to identify underlying disease mechanisms, specific biomarkers, and therapeutic targets for further testing in clinical trials.

Integration of large-scale multi-dimensional molecular and clinical data sets obtained from a well-characterized patient population could enhance our ability to understand the spectrum of molecular disease entities that present as a common histological phenotype, elucidate pathogenesis, and expedite scientific innovation toward safe and effective treatments for glomerular diseases[3].

To facilitate studies of this type, the Nephrotic Syndrome Study Network (NEPTUNE), supported by the National Institutes of Diabetes and Digestive and Kidney Disease (NIDDK), and the Office for Rare Diseases Research (ORDR) at the National Institutes of Health (NIH), and The NephCure and Halpin Foundations, was designed to conduct multi-disciplinary innovative research to address the "translational gap," which is a barrier to successful management of adults and children with FSGS, MCD and MN.

The overall objectives of the NEPTUNE network (Table 1) are incorporated into the NEPTUNE observational cohort study that is designed to address the following general and disease-specific hypotheses:

1. After accounting for types, duration, and frequency of immunomodulating therapy, the probability of remission of FSGS, MCD and MN, defined as change in urinary protein excretion and/or renal function, will be predicted by baseline demographics, clinical, genetic, and histopathological characteristics at the time of presentation.

2. Genome-wide molecular profiles obtained from renal biopsy tissue at the time of diagnosis of FSGS, MCD and MN will independently predict the proportion of subjects entering remission.

3. Individual genetic causes of FSGS and MCD activate specific transcriptional pathways in renal tissue and the molecular characterization of these transcriptional pathways will identify novel therapeutic targets yielding more complete and sustained disease remission.

4. The use of non-immunomodulating therapy (including anti-hypertensive, lipid-lowering, and antithrombotic agents and dietary manipulation) will have an independent effect on the probability of remission of nephrotic syndrome.

5. The composite rates of major medical complications (vascular thrombosis, complications of the kidney biopsy procedure, infection, and death) will vary according to the baseline characteristics and types of treatment in patients.

6. Nephrotic syndrome, its treatment and clinical outcomes, will have a varied measurable effect on quality of life of patients.

Results

Overview of Study Design and Organization

The NEPTUNE study is a prospective observational study that will enroll 450 children and adults with FSGS, MCD and MN at the time of first clinically-indicated renal biopsy for diagnosis of proteinuria. Enrollment from 18 clinical centers in the U.S. and Canada (Table 2) will occur over 30 months with a minimum follow-up of 30 months. The two primary

study outcomes are change in urinary protein excretion and change in renal function as indicated in Table 3. Secondary study outcomes are quality of life assessment, development of new-onset diabetes, malignancies, infections, thromboembolic events, hospitalization, acute kidney injury, and death.

The NEPTUNE consortium infrastructure consists of a Steering Committee, a Central Digitalized Histopathological Archive at the NIH, a Central Biorepository, Biochemical Laboratory and Data Analysis and Coordinating Center (DACC) at the University of Michigan, an Observational Study Monitoring Board at the NIDDK, and an external Scientific Advisory Board.

The overall design of the NEPTUNE cohort study is depicted in Figure 1 and described in detail below, NEPTUNE is registered at clinicaltrials.gov under NCT01209000.

NEPTUNE Cohort Participants

The NEPTUNE study will enroll a diverse population reflective of the disease burden in North America. Eligible candidates for the NEPTUNE study are individuals of any age scheduled for a clinically-indicated renal biopsy with proteinuria 500 mg/day estimated from a 24-hour or spot (protein to creatinine ratio) urine collection. Patients with sub-nephrotic proteinuria are included to capture the broad spectrum of clinical presentations[4, 5]. Exclusion criteria include patients with clinical, serological, or histological evidence of other renal diseases (e.g. diabetic nephropathy, systemic lupus erythematosus, etc), prior solid organ transplant, life expectancy < 6 months, and those who are unwilling or unable to consent. Cohort assignment is based on detailed renal histopathological analysis. Participants whose histopathology is not determined to be consistent with FSGS, MCD and MN are assigned to an "Other Glomerulopathies" cohort and retained in the study to serve as a comparison group and for future studies addressing these histopathologically-distinct entities. Eligible candidates over age 18 are enrolled after providing written, informed consent.

NEPTUNE Pediatric Population

Children are an important population affected by nephrotic syndrome (NS) and are enrolled in the NEPTUNE study at many clinical centers as indicated in Table 2. The NEPTUNE study will enroll eligible children after parental written consent (and child assent when appropriate) prior to the diagnostic renal biopsy. Information on medications prescribed for NS prior to enrollment will be collected in all participants including children who may have a longer exposure to pre-biopsy therapies consistent with standard pediatric nephrotic syndrome management[6]. Additional funding to enroll children from initial nephrotic syndrome presentation is currently being pursued.

Study Enrollment and Follow-Up Visits

The enrollment process includes a screening visit to assess eligibility and willingness to participate, a baseline visit for collection of clinical and demographic information, and a renal biopsy visit to obtain renal tissue samples. Renal biopsy material will be used to confirm assignment into the specific study cohort and for research purposes. The baseline

visit includes collection of a detailed medical history, sociodemographic information, physical measures, and blood and urine samples (see https://rarediseasesnetwork.epi.usf.edu/NEPTUNE/professional/studynetwork.htm# for details). In addition to age-appropriate questionnaires to assess quality of life, self-reported health will be evaluated using the Patient Reported Outcomes Measurement Information System (PROMIS, www.nihpromis.org). After enrollment, NEPTUNE participants are evaluated at 4-month intervals during the first year and every 6 months thereafter with similar data collection as performed in the baseline visit.

NEPTUNE Pathology Digital Imaging and Scoring System

Pathology material including glass slides, immunofluorescence images, and electron-micrographs, will be scanned into high resolution digital images and stored in the NEPTUNE pathology database along with copies of the local pathology reports. This database can be accessed remotely by the NEPTUNE Histopathology committee for review, scoring, and cohort assignment by disease classification.

The NEPTUNE Histopathology Committee has developed a novel strategy for scoring renal biopsies designed to standardize morphologic analysis[78]. The pathology protocol is designed to provide 1) multi-level annotation of glomeruli for multi-dimensional reconstruction to allow simultaneous review and scoring of the same glomerulus on each digital slide, and 2) identification and scoring of specific pre-defined morphologic "descriptors". This approach for kidney tissue analysis has the potential to redefine histopathologic classification and link these novel criteria to clinical and molecular phenotypes. In addition, the NEPTUNE pathology database will serve as a major resource available to investigators for future pilot and ancillary studies.

Overview of Applied Systems Biology Analysis Strategy

Systems biology is an emerging scientific approach that aims to address the 'pluralism of cause and effects in biological networks'[9] that defines the steady state and its disturbances in health and disease[10][11][12][13, 14]. The NEPTUNE study aims to integrate comprehensive clinical and pathologic phenotypes with extensive analysis of the molecular regulatory networks active in glomerular diseases. As depicted in figure 2, NEPTUNE will prospectively procure human samples for analyses focused on genome-wide profiling of genetic variation, epigenetic markers and transcriptomic, proteomic, and metabolomic profiles. Emerging systems biology approaches will be used to define associations between these multi-phasic and inter-related genome scale profiles and the detailed clinical and structural phenotypes.

The NEPTUNE study will establish a core molecular data set containing genetic profiles and renal tissue, compartment-specific, genome wide mRNA expression data to facilitate multi- and trans-disciplinary research exploration along the genotype – phenotype continuum[10][11][12][13].The NEPTUNE core data sets will provide a foundation to link mechanistic information with clinical disease presentation, and link molecular information from model systems with human nephrotic syndrome data sets. Patient subgroups with activation of specific molecular mechanisms can be used to assess the efficacy of novel

therapeutics in a highly targeted manner. Defining patients by the underlying disease mechanism will reduce the heterogeneity inherent to the current histology-based glomerular disease classification and, as a result, will focus the analysis and interpretation of clinical trials.

The core and ancillary data generated in NEPTUNE can be a significant resource to the renal research community. NEPTUNE will utilize Nephromine (www.Nephromine.org), a renal-specific systems biology search engine, to enable large-scale data analysis by nephrology investigators with and without systems biology expertise.

Statistical Considerations

The research aims will be explored separately in FSGS, MCD cohort and MN cohort. The primary objective of the statistical analysis plan is to build a predictive model that utilizes multiple sources of data, including molecular biomarkers and auxiliary predictors (e.g. race/ ethnicity, edema), to classify patients according to the proteinuria outcome (remission) as the primary endpoint. Secondary study endpoints include longitudinal measures of renal function. The primary analysis plan involves deriving a prediction model to rigorously evaluate and identify potential predictors for study endpoints. Subsequently, the overall prediction model will be used to assess relative risk and association via odds ratios for targeted sub-group analysis. In analysis of data from the network, random-effects models will be used to account for potential heterogeneity and unstructured interventions across and within the clinical centers. Also, the analysis is tailored to handle dropouts, incomplete longitudinal data, and other types of missing data[15]. For further details on statistical methodology, see Supplement.

For biomarker discovery and validation, the sample will be split into the training and the validation sets at the ratio of $\frac{2}{3}$n:$\frac{1}{3}n$[16, 17]. With regard to the primary endpoint, selection of biomarkers (e.g. gene expression levels or serum or urine proteomic markers) will proceed in two steps: first, univariate variable screening will identify a small pool of promising biomarkers and, subsequently, joint screening to narrow identified candidate biomarkers to those with the optimal predictive power. Using selected biomarkers, a prediction model will be developed that is further validated via the test cohort by the criteria of ROC curves and net reclassification index[18, 19]. In the analysis of secondary endpoints, similar strategies will be applied to build prediction models to classify longitudinal trajectories (e.g. estimated GFR).

Sample Size Consideration

The NEPTUNE study will have adequate power to identify important molecular biomarkers predicting the primary endpoints. Power calculations were based on the probability of correct classification (PCC)[20]. The expected probability of PCC is a weighted average of sensitivity (probability o f remission) and specificity (probability of non-remission). An estimate 20,000 genes (probes) and 75 auxiliary predictors will be screened to identify true gene expression markers and true auxiliary predictor for the defined endpoints (Table 4). The PCCs are 0.874, 0.942 and 0.966 for the sample sizes of 100, 150 and 200, respectively,

based on the identification of 50 true gene expression markers and 10 true auxillary markers. The NEPTUNE study enrollment goal is to enroll 450 subjects.

NEPTUNE Training, Ancillary Study and Outreach Program

In the spirit of accelerating NS research, NEPTUNE has organized a pilot and ancillary studies program to facilitate high-quality investigation within the research community utilizing the resources provided by the NEPTUNE infrastructure. The ancillary studies mechanism is open to investigators regardless of institutional affiliation. Details on the available data, specimens and process required to gain access to these resources are provided at https://rarediseasesnetwork.epi.usf.edu/NEPTUNE/professional/studynetwork.htm#. Further, NEPTUNE supports a training program for post-doctoral fellows and junior faculty to promote career development in clinical and translational research in glomerular disease (https://rarediseasesnetwork.epi.usf.edu/NEPTUNE/professional/fellowship/). Lastly, NEPTUNE has organized a contact registry within the Rare Diseases Clinical Research Network (www.neptune-study.org) for patients with NS. This registry contains self-reported information, including diagnostic, demographic, and contact information that can be used for patient education and to facilitate enrollment of patients into clinical trials and other research studies. At the time of this publication, over 1300 patients are actively enrolled in the NEPTUNE contact registry and a substantial number of registrants have provided consent to be directly approached for participation in clinical studies.

Discussion

Primary NS is a debilitating clinical entity affecting individuals of all ages and associated with end-stage renal disease and premature death. We remain limited in our ability to effectively treat the specific diseases presenting as NS due to our lack of understanding of disease mechanisms and lack of predictors to identify clinical course and therapeutic responsiveness. The NEPTUNE cohort study is designed to address these gaps in knowledge in prominent NS disorders, FSGS, MCD and MN, by developing a platform for comprehensive longitudinal assessment of patients with incipient disease. This study represents strong commitment to bring together expertise from a broad range of disciplines focused on large-scale integration of clinical, histopathologic and molecular data. This state-of-the-art approach to identify pathogenic mechanisms and novel biomarkers has the greatest potential to advance medical management of these complex diseases.

Epidemiologic data demonstrates an increased incidence of primary NS due to FSGS over several decades in adults and children[21]. In young children, MCD remains the predominant etiology of primary NS. However, 10–15% of primary NS is caused by MCD in adults. FSGS and MCD are often considered related entities due to their similarities in clinical presentation, renal pathologic features as well as common genetic polymorphisms[22, 23], but a systematic comparative analysis in sporadic patients is lacking. Although the clinical course can be highly variable, patients with FSGS are more likely resistant to glucocorticoid therapy and have greater risks for kidney failure. A variety of monogenetic mutations (both dominant and recessive patterns) with distinct functional consequences have been identified that present with indistinguishable FSGS-type lesions signifying heterogeneity in disease

pathogenesis[22, 24–29]. Therefore, it is evident that the current classification and diagnostic tools are insufficient in guiding therapy. The paucity of conclusive clinical interventional studies for glomerular disease in general underscores the need to establish predictive biomarkers and therapeutic targets for future trials in molecularly-defined NS cohorts[30].

Recent discovery of genetic risk loci in linkage disequilibrium with *APOL1 and MYH9*[31–33] among people of African descent with FSGS (and potentially other kidney disease) is a major step toward understanding racial/ethnic disparities in kidney disease. Recruitment of a diverse study population from sites located across North America provides opportunities to explore, in a longitudinal fashion, the clinical manifestations and therapeutic implications of these and other genetic variants.

MN is a common cause of primary NS in adults[34, 35] and has a highly variable course from spontaneous remission to progression to end-stage renal disease. As with FSGS and MCD, renal tissue classification is descriptive and not associated with clinical course. The clinical course is highly variable and difficult to predict at presentation often leading to prolonged observation of patients by clinicians to determine prognosis and guide therapy[36, 3738]. Recent evidence suggests that circulating phospholipase A2 receptor autoantibodies may be an immunologic marker for the majority of cases of primary MN[39, 40]. A major emphasis for the MN cohort in NEPTUNE is to understand the value of these new biomarkers in predicting disease progression and response to treatment.

In line with the major goals of the consortium, the NEPTUNE pathology protocol for scoring renal biopsies has the potential to re-define morphologic descriptors utilizing innovative digital methodology and serve as an extensive resource for future ancillary studies. This approach to renal biopsy analysis has potential applicability for risk stratification, disease classification and clinical trial design.

There is rapid expansion of information about monogenetic diseases and genetic loci associated with NS. However, discovering the functional links that bridge the gap from genetic risk to disease phenotype is one of the main challenges ahead. The NEPTUNE cohort study has been designed to bridge this genotype-phenotype gap using the integrated approaches of systems biology. These emerging innovative strategies will be the first steps towards identifying and testing rational interventions in primary NS. Overall, the NEPTUNE study and its complementary programs provide unparalleled opportunities to make fundamental observations using state-of-the-art research methodology, foster collaborative scientific activity, and facilitate research career development to stimulate progress in the management of glomerular diseases.

Methods

See supplement for a more detailed definition of statistical methodology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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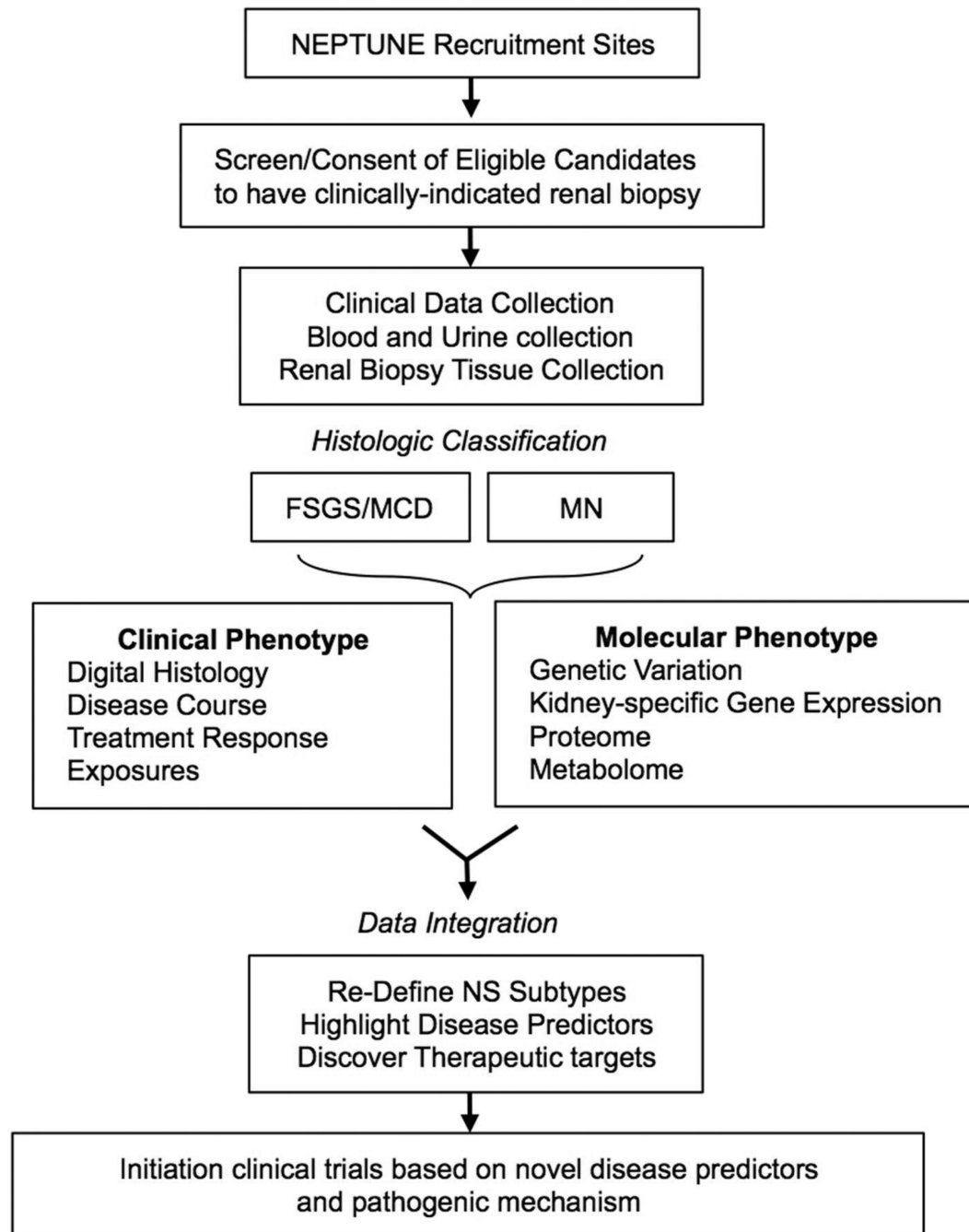
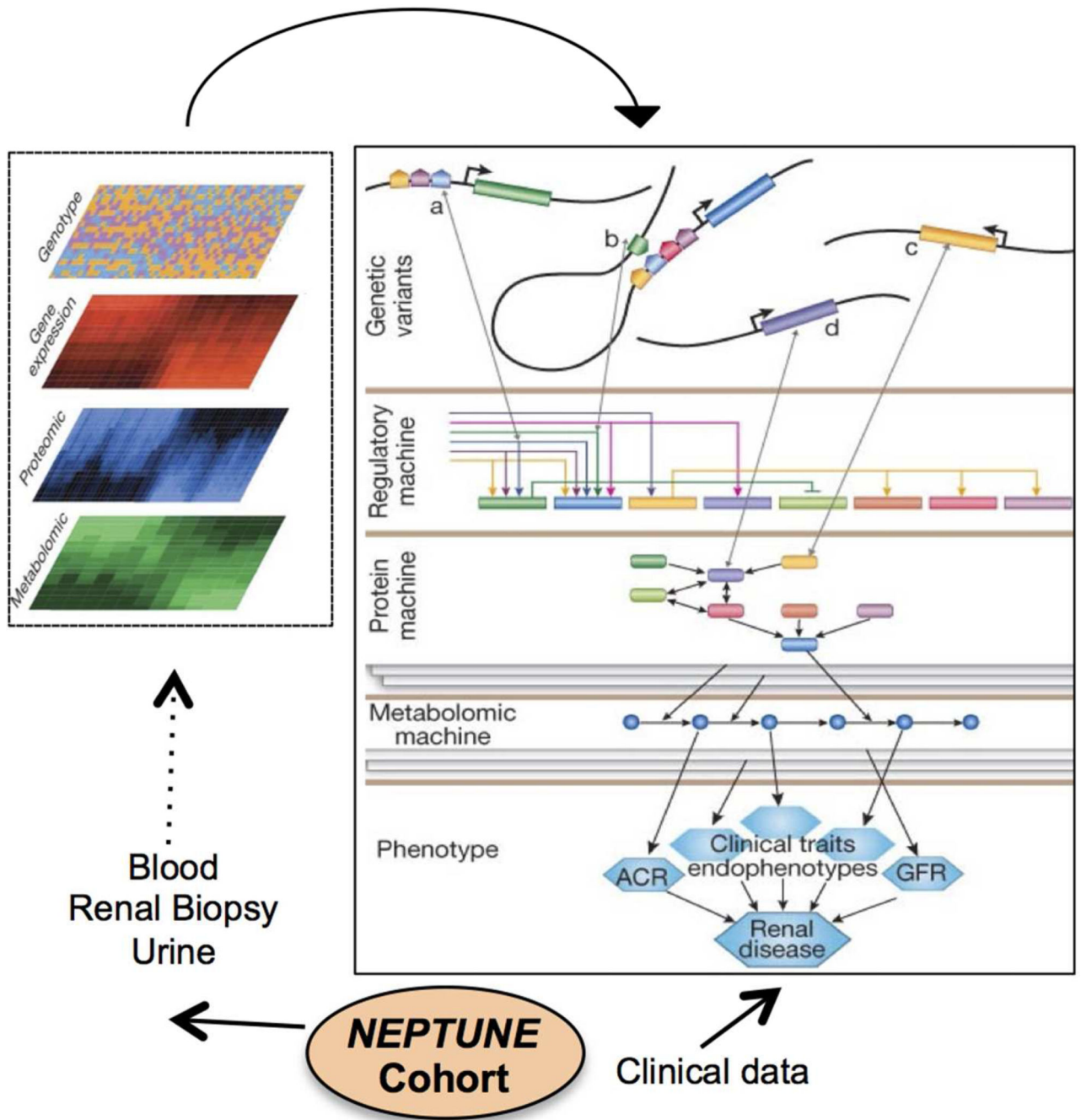**Figure 1. NEPTUNE Cohort Study Design**

Figure 2. Overview of Multilevel Data Integration in NEPTUNE

"The integrative analytical approach in NEPTUNE relies on obtaining comprehensive molecular and clinical information from each subject. For molecular analysis, the initial step is to generate large-scale datasets of genome-wide genetic variation and targeted gene sequencing, compartment specific gene expression data on renal biopsy tissue, leukocytes and urine, and proteomic/metabolomic data from both urine and blood samples. Each molecular dataset will be interrogated alone and in relationship with the others in order to understand multidimensional molecular interactions. In addition, the molecular data will be

analyzed with the comprehensive clinical information to determine associations between molecular events and clinical outcomes. The overall goal is to develop a framework for studies to define the molecular heterogeneity of NS for disease stratification, biomarker identification and molecular target definition. (Figure modified from Keller et al[10])."

Table 1

Objectives of the Nephrotic Syndrome Study Network (NEPTUNE)

Objectives of the Nephrotic Syndrome Study Network
Establish a collaborative, integrated, cost-effective investigational infrastructure to conduct clinical and translational research in FSGS, MCD, and MN
Perform a longitudinal observational cohort study on patients with incipient biopsy proven NS
Establish Pilot and Ancillary Projects Programs using the unique resources, clinical data, or specimens collected by NEPTUNE
Establish a Training Program for post-doctoral/junior faculty trainees to prepare for clinical and translational research in glomerular disease
Collaborate with the ORDR Data Management and Coordinating Center and the NephCure Foundation to establish a web-based exchange platform for lay people, physicians, and scientists.

Table 2

Nephrotic Syndrome Study Network Clinical Centers

NEPTUNE Clinical Centers[*]	Recruitment Population
Case Western Medical Center, Cleveland, OH	Adult and Pediatric
Cohen's Children's Medical Center, Manhasset, NY	Pediatric
Columbia University, New York, NY	Adult
Emory University, Atlanta, GA	Pediatric
Harbor Biomedical Research Institute, Torrance, CA	Adult
Johns Hopkins Medical Center, Baltimore, MD	Pediatric
Montefiore Medical Center, Bronx, NY	Adult and Pediatric
The Mayo Clinic, Rochester, MN	Adult and Pediatric
National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD	Adult
New York University School of Medicine, New York, NY	Adult and Pediatric
Temple University Health Systems, Philadelphia, PA	Adult
University Health Network, Toronto, Canada	Adult
University of Michigan Health Systems, Ann Arbor, MI	Adult and Pediatric
University of Miami Medical Center, Miami, FL	Adult and Pediatric
University of North Carolina, Chapel Hill, NC	Adult and Pediatric
University of Pennsylvania, Philadelphia, PA	Adult and Pediatric
University of Southern California Children's Hospital, Los Angeles, CA	Pediatric
University of Washington, Seattle, WA	Adult and Pediatric

[*] For full list of sites enrolling NEPTUNE participants, see Appendix

Table 3

NEPTUNE Primary Outcome Variables and Definitions

Rate of Change in Urinary Protein Excretion from Baseline (UP:C ratio)			Rate of Change in Renal Function from Baseline
Definitions	**Standard**	**Cohort Study**	**Cohort Study**
Reduction in UP:C ratio of 0.3 Reduction in UP:C ratio > 50% and final U:C ratio 0.3 and 3.5	Complete Remission Partial Remission	Complete/Partial Remission	25 ml/min/1.73m^2 reduction in eGFR*
Reduction in UP:C ratio > 50% and final >3.5 Reduction in UP:C ratio < 50% (includes increases < 50%) Urine P:C ratio increases by >50%	Limited Response Non-Response Progressive Proteinuria	No Response	50% decline in eGFR from baseline
New development of UP:C ratio >3.5 after complete or partial remission	Relapse	Relapse	ESRD (initiation of dialysis, kidney transplantation or eGFR 10 ml/min/1.73m^2

"Abbreviations: UP:C = urine protein: creatinine (g/g); eGFR = estimated glomerular filtration rate; ESRD = end stage renal disease"

Table 4

Sample size and Classification Power for the NEPTUNE Study

		Prevalence = 0.6					Prevalence = 0.3			
		$r_m = 5$	$r_C = 10$				$r_m = 5$	$r_C = 10$		
			PCC					PCC		
δ_m/σ_m	PCC*	n=100	n=150	n=200	PCC*		n=100	n=150	n=200	
0.2	0.788	0.638	0.667	0.687	0.812		0.670	0.675	0.690	
0.3	0.881	0.776	0.803	0.818	0.892		0.779	0.807	0.822	
		$r_m = 50$	$r_C = 10$				$r_m = 50$	$r_C = 10$		
			PCC					PCC		
δ_m/σ_m	PCC*	n=100	n=150	n=200	PCC*		n=100	n=150	n=200	
0.2	0.941	0.639	0.695	0.753	0.948		0.694	0.697	0.754	
0.3	0.990	0.866	0.938	0.964	0.991		0.867	0.939	0.964	

"Sample size and power determination are based on a classification model for the primary endpoints with significant predictors. PCC* is the maximal classification power that would be obtained under r_m genetic predictors (of 20,000 genes) and r_C clinical predictors (of 75 clinical parameters) with respect to the standardized effect-sizes, δ_m/σ_m, where δ_m and σ_m are the effect size and standard deviation of an important biomarker, respectively. In parallel, PCC is the achievable power in the actual study with n patients enrolled in the FSGS, MCD, or MN subcohorts in NEPTUNE."