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Title: Identification and Functional Characterization of Novel Genes Mutated in Nephrotic Syndrome

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Abstract

Nephrotic syndrome (NS) is one of the most frequent and an intractable cause of chronic kidney disease (CKD), which, in turn, represents one of the largest health burdens in India and other countries, and is continuously increasing for unknown reasons. It is a disease of kidney glomerular filtration barrier characterized by gross proteinuria with hypoalbuminemia, edema and dyslipidemia. In the last 15 years, about 37 recessive and 8 dominant genes have been discovered to cause NS in humans, if mutated. No efficient treatment exists for NS and its disease mechanisms remain obscure. Incidence of NS in India is very high (~7 per 1,00,000 children).However, to date, no genetic study has been conducted to study the specific alleles and genes mutated in Indian population, thus causing NS. We hypothesized thatcombining homozygosity mapping (HM) and whole exome resequencing (WES) can facilitate the identification of mutations of novel single-gene causes of NS, specifically in Indian population where no genetic study is conducted so far.

To study the genetic cause of this disease in India, we selected and recruited of patients and collected blood samples and pedigrees from Indian patients with NS in collaboration with Prof. Arvind Bagga, Dept. of Nephrology, AIIMS, New Delhi. From Oct 2015 to July 2017, we screened 1,150 individuals with kidney disorder for this study. After applying all the inclusion and exclusion criteria, we obtained blood

samples and pedigrees following informed consent from ***96 individuals with NS***. In total, we solved **17** out of 96 individuals (17.7%) with mutation in one of the known SRNS gene (*Nephrin, Podocin, SMARCAL1, LAMB2* or *PLCE1*) by sanger sequencing and high-throughput exon sequencing. This is the first study in India uncovering the genetic causes and specific variants involved in the pathogenesis of NS in India. The remaining 79 unsolved caseswere then submitted for whole exome sequencing.

To identify novel genes mutated in NS we performed HMcombined with WES in 79 remaining individuals from India. We also performed high-throughput exon sequencing in a worldwide cohort of ~1,000 additional families with NS, examining specific candidate genes for NS based on genetic mouse models of NS. By using WES, we identified *TNS2*(*tensin-2*) as the first gene that can cause partially treatment sensitive NSin the Indian population and worldwide. Additionally, we discovered mutations in 4 other genes (*MAGI2, DLC1, ITSN1,* and *ITSN2*) as causing NS in many families with partially treatment sensitive NS. Importantly, the proteins physically or functionally interact to regulate RhoA or Cdc42 (and not Rac1).These patients have treatment-sensitive NS, revealing a functional module of treatment responsiveness for NS.This allows us to now study the therapeutic mechanisms of steroids on this network.Furthermore, in three unrelated families with steroid resistant NS, we discovered recessive mutations of advillin(*AVIL*), a member of the gelsolin superfamily of actin binding proteins.Immunofluorescence in human podocytes showed the truncating mutant allele of AVIL caused mislocalization with F-actin.We identified PLCε1 as new interaction partners of advillin and characterized its role for actin bundling properties. In summary, we also discovered that AVIL loss of function can cause SRNS and defined its role with the established SRNS protein PLCE1.