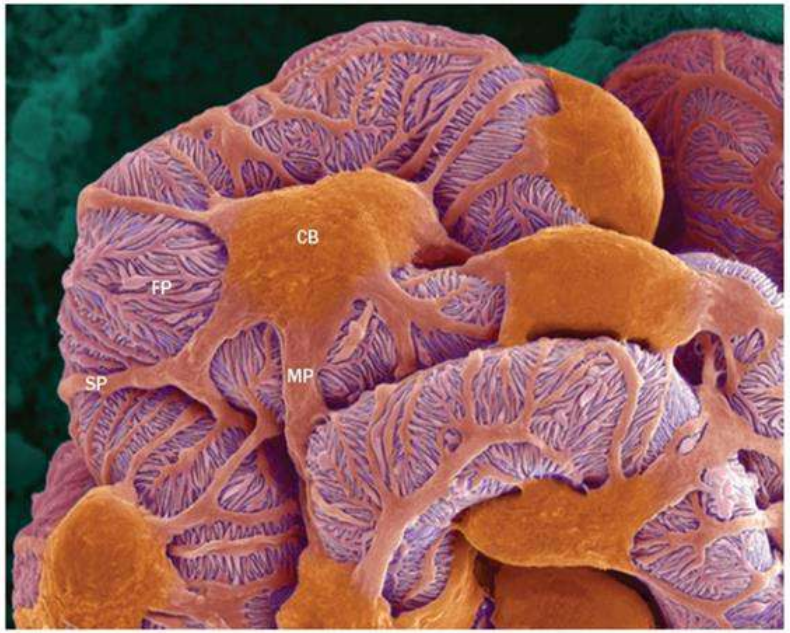

Nephrotic Syndrome:Immune Dysfunction or Podocytopathy

Anil Vasudevan

Professor and Head
Department of Pediatric Nephrology
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Division of Molecular Medicine
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IPNA-AsPNA Junior Master Class, New Delhi
26-27 November 2022

MEDICAL PROGRESS

THE "NEPHROTIC SYNDROME"*

STANLEY E. BRADLEY, M.D.,† AND CORNELIUS J. TYSON, M.D.‡

the absence of renal disease as so-called "pure," "genuine" or lipoid nephrosis. A prolonged debate has centered about this entity. It is claimed on the one hand that the disorder is renal in origin, either a disease entity *sui generis*[2, 3] or the result of an unrecognized glomerulonephritis,[4, 5] and, on the other, that it is primarily extrarenal, perhaps on the basis of some obscure derangement of protein metabolism.[6, 7] Conflicting opinions regarding the pathogenesis of various manifestations are likewise unsettled, but there is general agreement that the

concluded that "The nephrotic syndrome can be profitably viewed as a discrete entity. As such it remains an unsolved riddle."

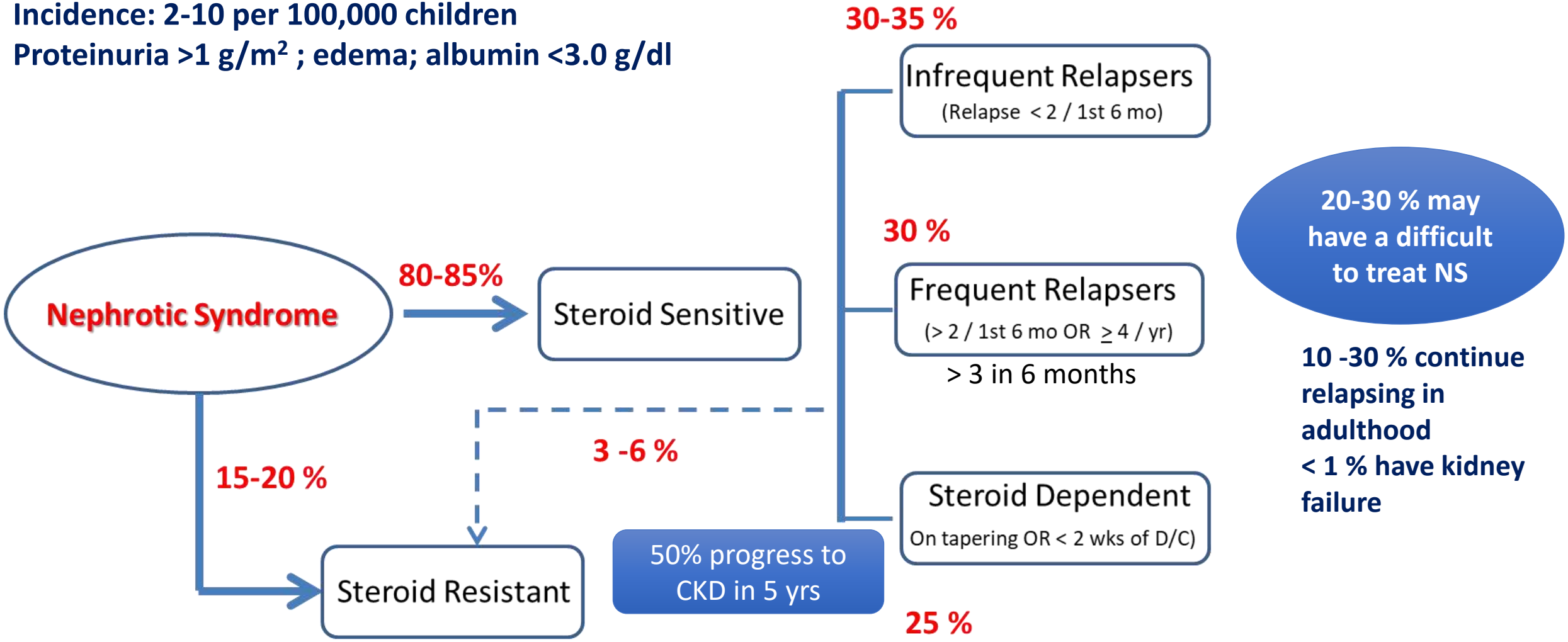
Bradley, S. E. and Tyson, C. J. The nephrotic syndrome, *New England J. Med.*, 1948, *238*:223, and 260.

Learning Objectives

- Recent advances in the understanding of immune dysfunction in idiopathic nephrotic syndrome
- Role of circulating permeability factors in pathogenesis of idiopathic nephrotic syndrome
- Podocytes as a key player in pathogenesis of nephrotic syndrome
- New insights in the genetics of nephrotic syndrome

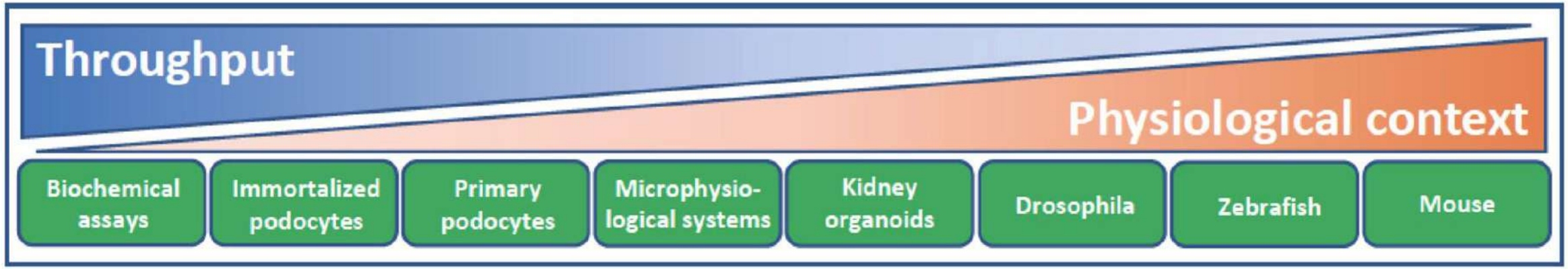
Natural History of NS

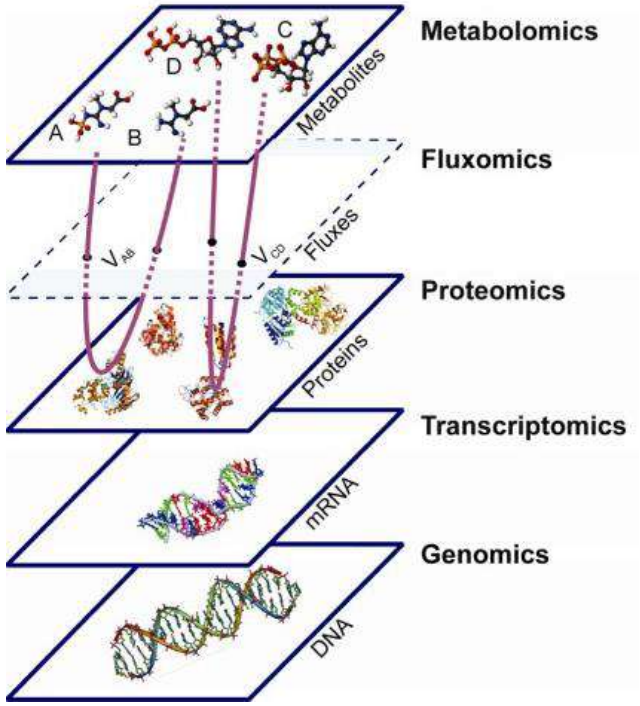
Incidence: 2-10 per 100,000 children
Proteinuria >1 g/m² ; edema; albumin <3.0 g/dl



Saleem et al, 2022 Indian Pediatr. 2012, Curr Opin Pediatr 2008

Tools to help better understand NS





Multilayered omics approaches

Registries and Cohorts

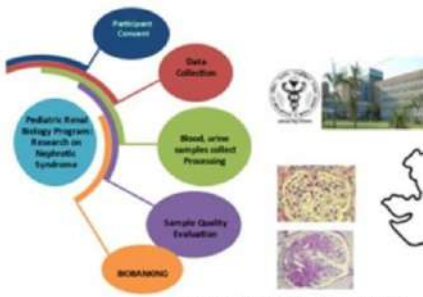
neptune

Observational Studies during clinical care

the NEPHROVIR population-based cohort study

INSIGHT INTO NEPHROTIC SY

NEPHQUEST - H
based cohort st

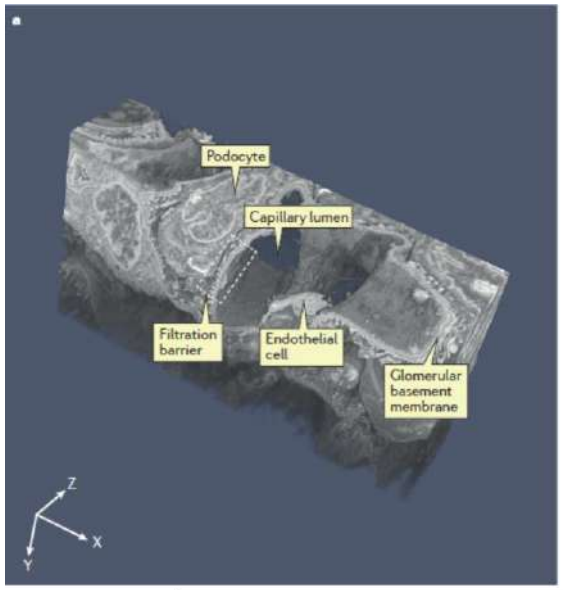


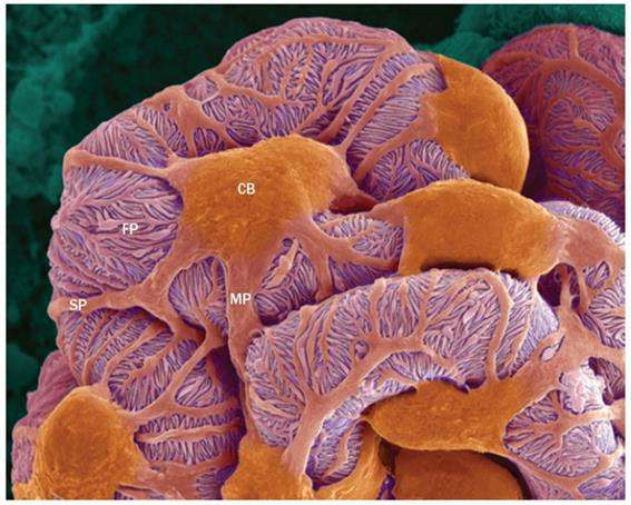
Biospecimen aliquots per visit

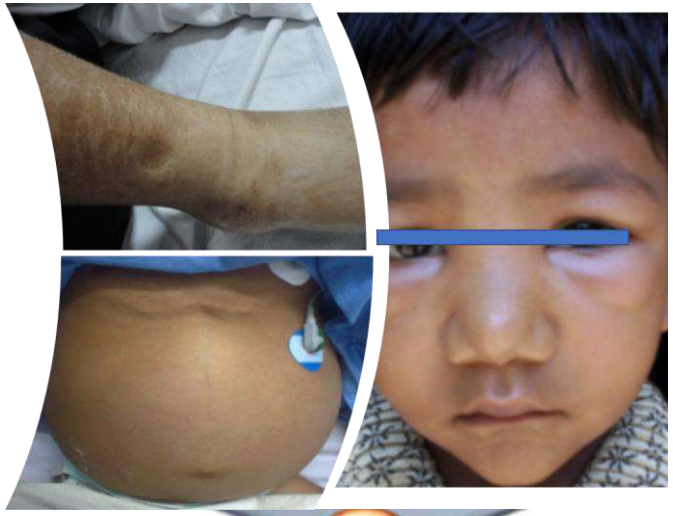
Courtesy Aditi Sinha

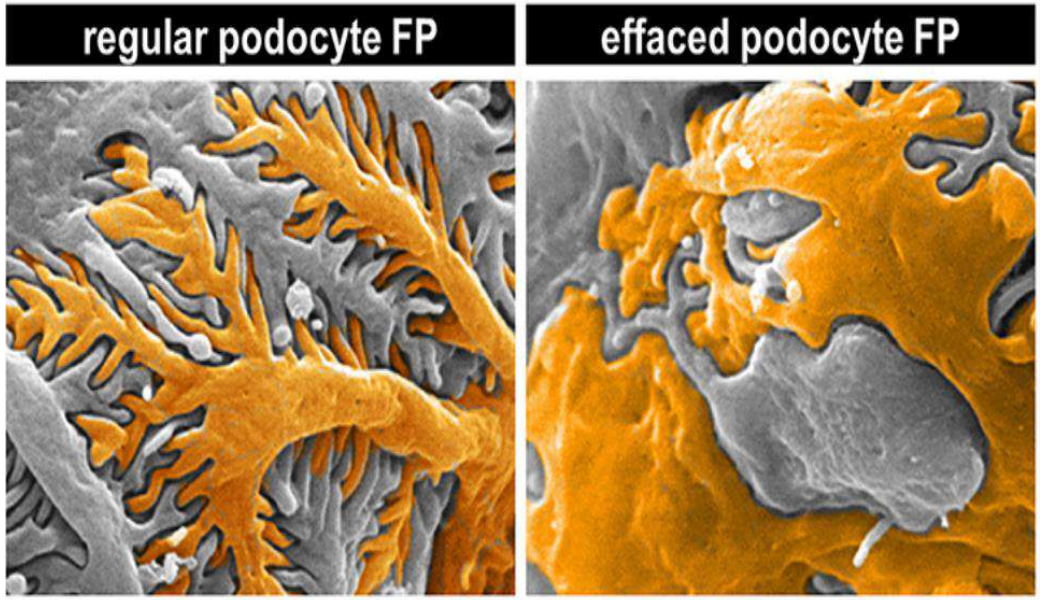
Nephrotic Syndrome is a podocytopathy

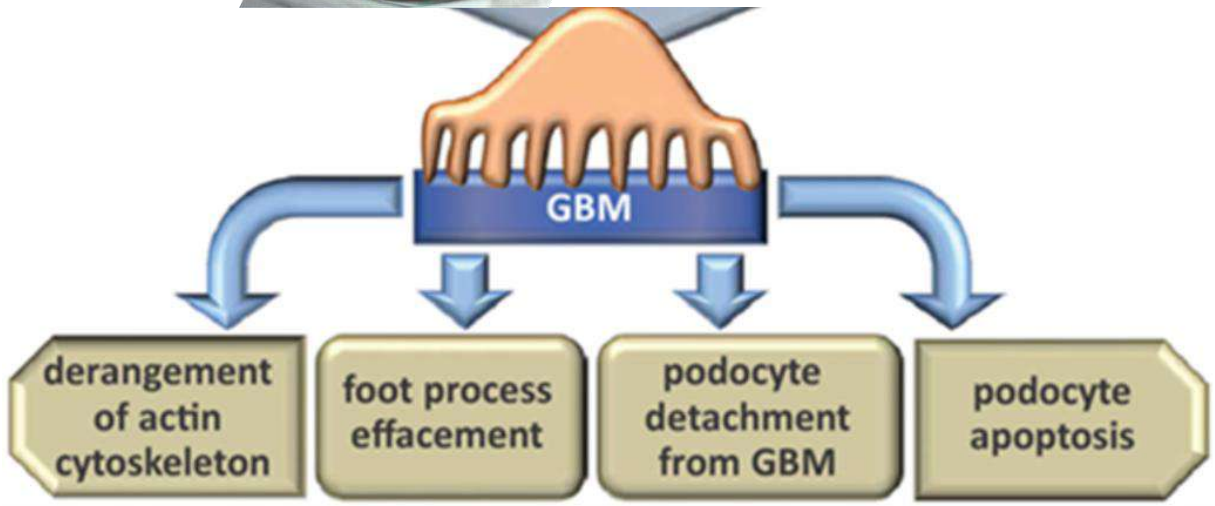
Podocytes are terminally differentiated cells











Nat Rev Nephrol. 2016 ;12: 692-710; Clin J Am Soc Nephrol 2: 529-542, 2007 Sci Rep 5, 8993 (2015)

Immune dysfunction

PATHOGENESIS OF LIPOID NEPHROSIS: A DISORDER OF T-CELL FUNCTION

ROBERT J. SHALHOUB

Veterans Administration Hospital, 50 Irving Street N.W., Washington D.C. 20422, U.S.A.

The purpose of this paper is to develop the following hypothesis: L.N. is a systemic disorder of C.M.I. in which episodic or sustained domination of the immune system by a clone of T cells results in the production of a circulating lymphokine toxic to the G.B.M. This lymphokine, tentatively named basement membrane toxin (B.M.T.), augments the permeability of G.B.M. to protein, culminating in a nephrotic syndrome. This pathogenetic sequence is inferred from four well-established clinical observations: (1) remission of L.N. associated with measles; (2) susceptibility to pneumococcal infections; (3) remissions induced by steroids and prolonged by cyclophosphamide; and (4) occurrence of similar glomerular changes in Hodgkin's disease.

Infusion of supernatants of cultured PBMC from patients with MCNS relapses induced proteinuria in rats

T cell hybridomas obtained from a patient with NS secreted a factor that caused proteinuria in rats

T cell transcriptome analysis, indicated a likely a thymic disorder

Lancet 1974;ii: 556–560

Is there a trigger for immune dysregulation in NS?

- 50 -60 % of relapses triggered by upper respiratory tract infection (Mostly viral)
- Increasing corticosteroid treatment during upper respiratory tract infections has been shown to decrease the likelihood of relapses
- Vaccination and atopy have also been associated with relapse

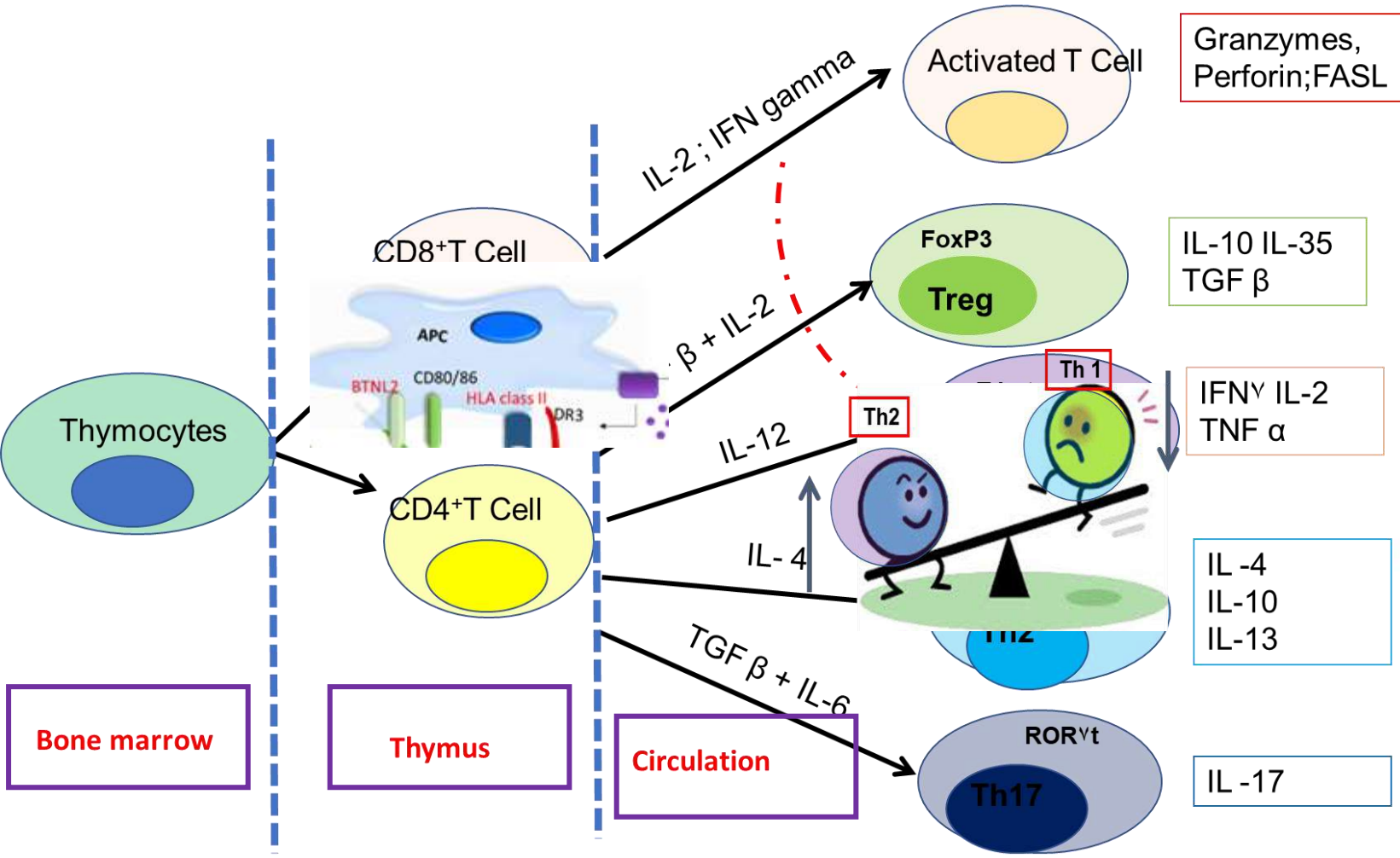
Virus	DNA detection and serology		Cases $N=124$ N (%)		Controls $N=196$ N (%)		OR [95%IC]	p
EBV	DNA	−	61	(49.2) (50.8)	139	(70.9) (29.1)	1 2.6 [1.6; 4.2]	0.0002
		+	63		57			
	IgM VCA	−	80	(64.5) (16.9) (18.6)	139	(70.9) (7.1) (21.9)	1 2.3 [1.1; 4.7]	0.02
		+ missing data	21		14 43		1.0 [0.5; 1.9]	ns
			23					
	IgG VCA	−	52	(41.9) (46.0) (12.1)	98	(50.0) (40.3) (9.7)	1 1.4 [0.9; 2.3]	ns
		+ missing data	57		79		1.9; [0.8; 4.2]	ns
			5		19			
	IgG EBNA	−	61	(49.2)	106	(54.1)	1.2 [0.7; 2.0]	ns
		+ missing data	49	(39.5)	72	(36.7)	1.7 [0.7; 4.0]	ns
			14	(11.3)	18	(9.2)		
CMV	DNA	−	110	(88.7)	189	(96.4)	1	0.0217
		+	14	(11.3)	7	(3.6)	2.9 [1.2; 7.4]	
	IgM	−	67	(54.0)	127	(64.8)	1	0.01
		+	14	(11.3)	7	(3.6)	3.4 [1.3; 9.2]	ns
		missing data	43	(34.7)	62	(31.6)	1.3 [0.8; 2.2]	
HHV-6	DNA	−	56	(45.2)	76	(38.8)	1	ns
		+	68	(54.8)	120	(61.2)	0.7 [0.5; 1.2]	

EBV, Epstein–Barr virus; *CMV*, cytomegalovirus; *HHV-6*, human herpesvirus-6; *HHV-7*, human herpesvirus-7; or. odds ratio

EBV Hypothesis

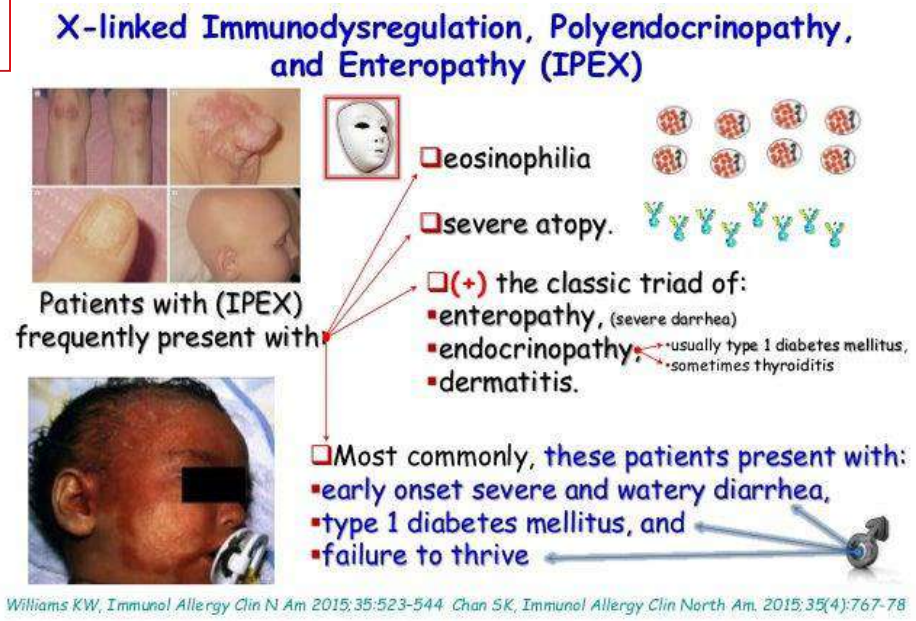
Am J Kidney Dis 2009;54:945–53; J Epidemiol 2015;25:370–7 Pediatr Nephrol 2014;29:2325–31

Role of T cell



Th 2 Bias – Hygiene hypothesis

Implication of Treg



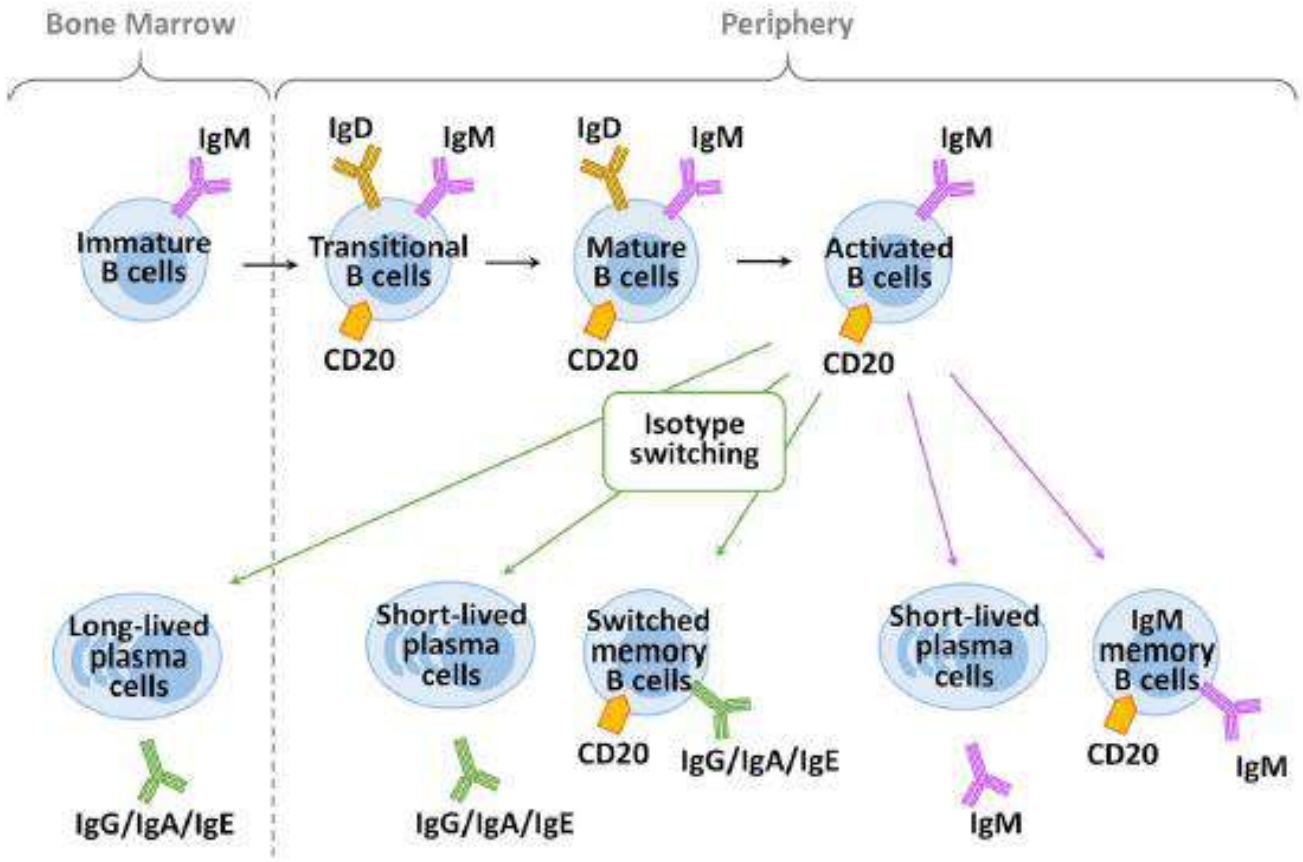
Experimental models support the association between low Tregs during a trigger event and proteinuria

Treg dysregulation can amplify the neutrophil-induced oxidative burst

J Am Soc Nephrol 2015; 20:57–67; Pediatr Int 2015; 57:e59–e6.

B Cells in NS

dysregulated immunoglobulin metabolism in INS
Association with Hodgkin's lymphoma – derived from B cell – EBV infection



Kerstin Benz · Jörg Dötsch · Wolfgang Rascher · Daniel Stachel

Change of the course of steroid-dependent nephrotic syndrome after rituximab therapy

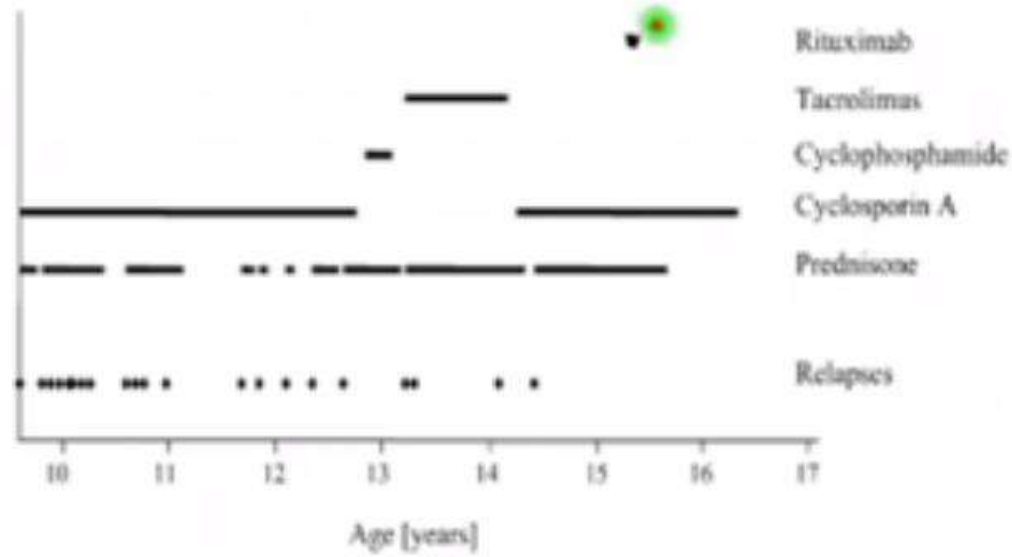
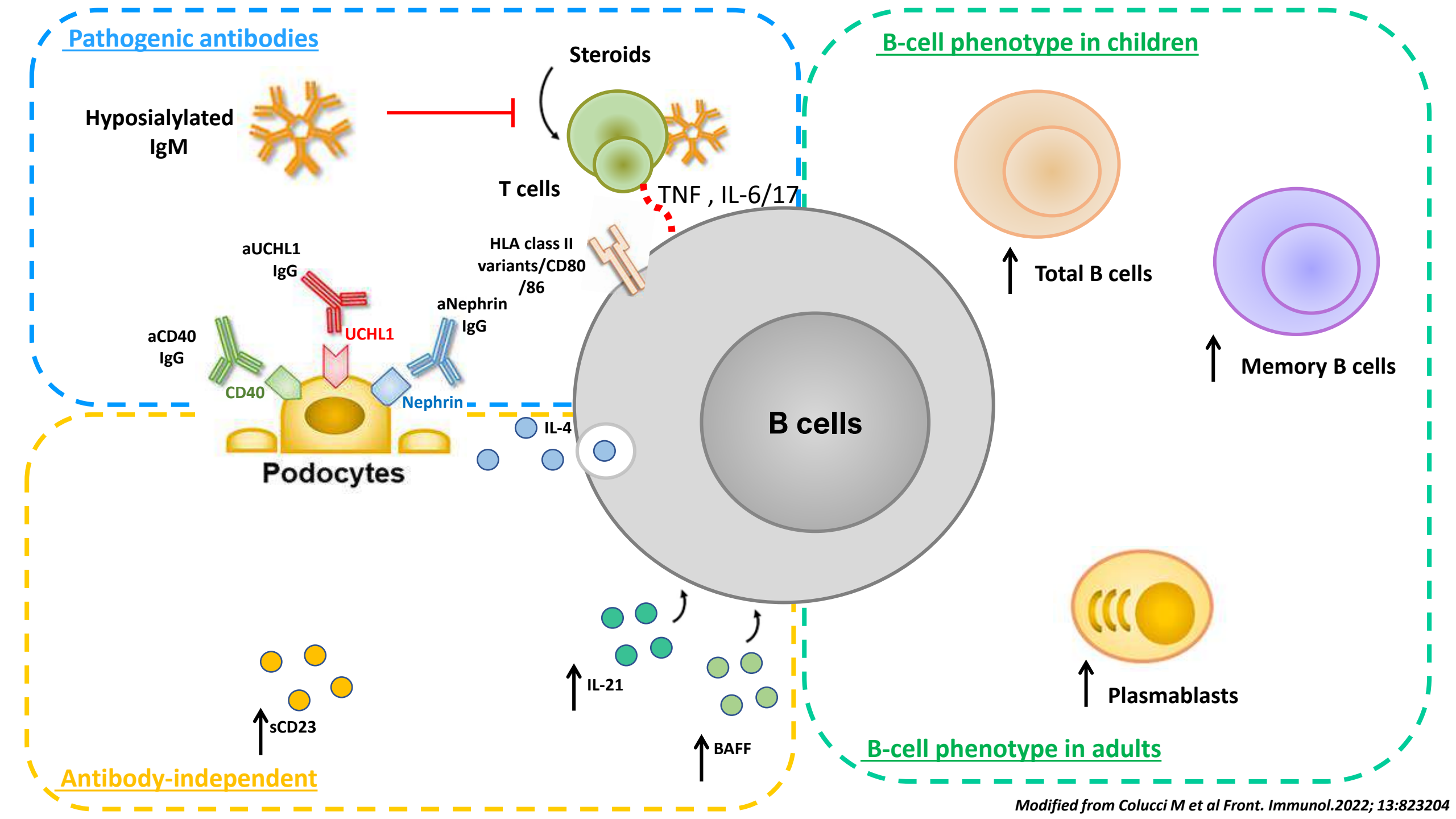
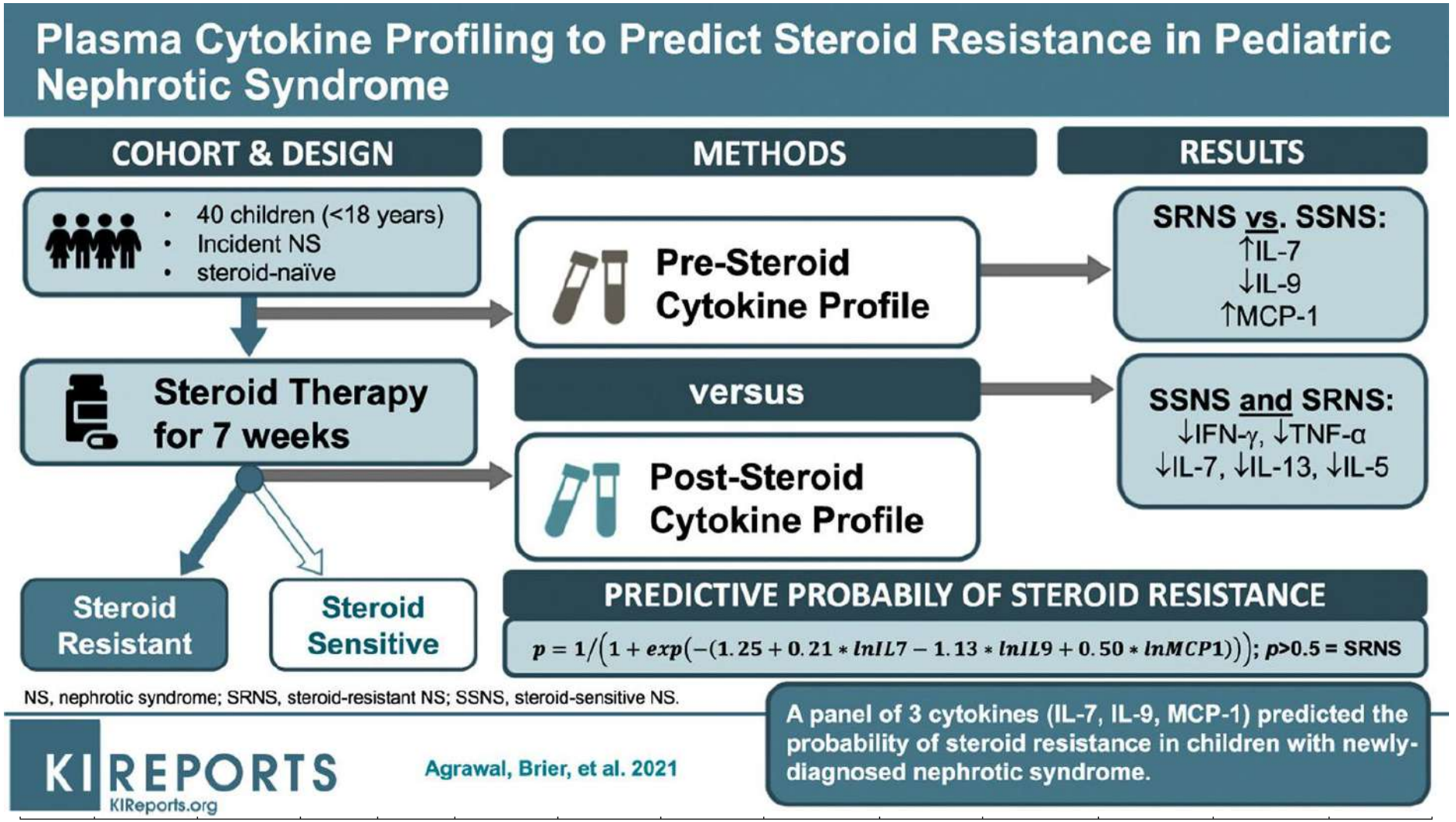


Fig. 1 Different treatments and relapses of steroid-dependent nephrotic syndrome in our patient between the age of 10 and 17 years

Colucci et al Pediatr Nephrol (2018):573–84


Pathogenic antibodies
Steroids
Hyposialylated IgM
T cells
TNF , IL-6/17
HLA class II variants/CD80 /86
aUCHL1 IgG
UCHL1
aNephrin IgG
aCD40 IgG
CD40
Nephrin
Podocytes
B cells
IL-4
B-cell phenotype in children
Total B cells
Memory B cells
Plasmablasts
B-cell phenotype in adults
IL-21
BAFF
sCD23
Antibody-independent
Modified from Colucci M et al Front. Immunol.2022; 13:823204

Immune factors: Causal, Consequence or Unassociated?



Circulating factors

RECURRENCE OF IDIOPATHIC NEPHROTIC SYNDROME AFTER RENAL TRANSPLANTATION

JOHN R. HOYER LEOPOLDO RAIJ
ROBERT L. VERNIER RICHARD L. SIMMONS
JOHN S. NAJARIAN ALFRED F. MICHAEL

Departments of Pediatrics, Internal Medicine, and Surgery, University of Minnesota Medical School, Minneapolis, Minnesota 55455, U.S.A.

urine does not clear of protein and these patients progress to renal failure. We have studied four such patients at the onset of their disease and after renal transplantation. The nephrotic syndrome recurred in three of them shortly after renal transplantation.

Case-reports

FIRST CASE

This boy developed intermittent periorbital œdema at 7½ years of age. 6 months later the nephrotic syndrome was diagnosed (fig. 1). Prednisone 80 mg. per day for 21 days

Trans-placental transmission of a “permeability factor” leading to neonatal transient proteinuria

Disease resolution when transplanted kidneys are removed and implanted in a different recipient

Serum from patients with post-transplant relapse of INS induce proteinuria in rats

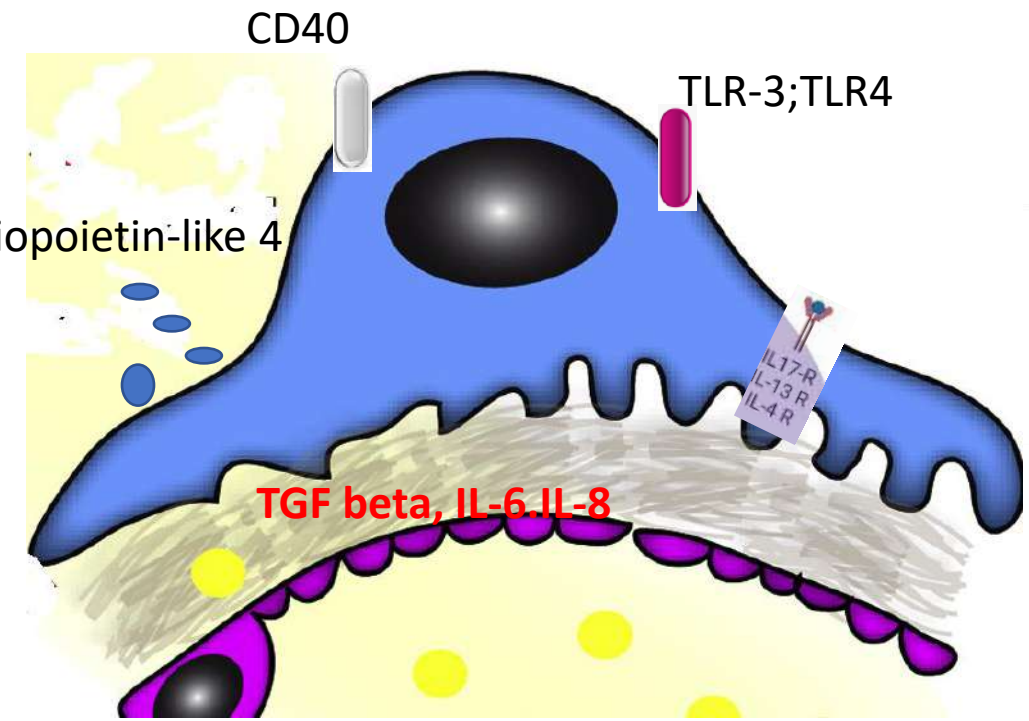
Lancet, 1972, ii, 343.

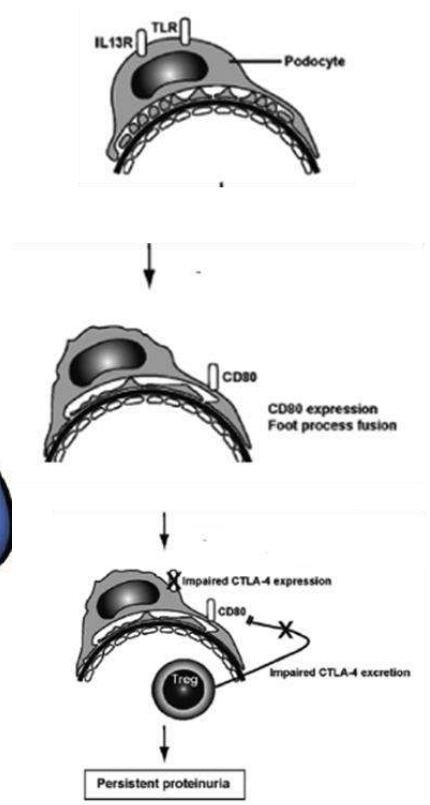
Circulating Factors – The Holy Grail

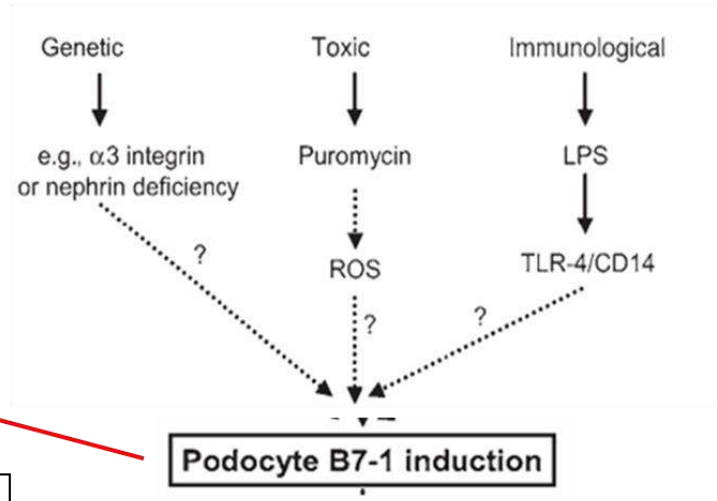
Putative permeability Factors	Molecular weight	Comments
VPF/GPF-Non-specific	60-160 kDa	Lagrue et al used isolated lymphocytes and cell culture supernatants caused capillary permeability in guinea pig skin Obtained from T-cell hybridoma made from patients with MCNS, induced proteinuria when injected into rats
Haemopexin	80-85 kDa	Both recombinant and human haemopexin induced reversible proteinuria accompanied by FPE in rats No validation studies have been performed
Interleukin 13/ Interleukin 8	16kDa	Increased expression of mRNA and cytoplasmic IL13 in CD4+/CD8+ T cells from children with steroids – sensitive NS. Overexpression of IL13 in rats induces MCNSs-like disease. Il-8 increased in MCNS relapses. No clinical studies
CLC-1	22-25kDa	Increased glomerular permeability, decreased nephrin expression in cultured podocytes Antibody to CLD-1 reverse the permeability effect of FSGS sera
suPAR	20-50 kDa	Activated podocyte β3 integrin, resulting in reorganization of the actin cytoskeleton of podocytes Experimental data were not supported by clinical data
Reactive Oxygen Species **Oxidized Albumin**		Increased ROS generation and decreased antioxidant defence in NS plasma Puromycin and adriamycin induced NS in rats demonstrate ROS related damage NO prevents the increase of permeability to albumin induced by the TNF alpha-inducedO^{2-} production 10 increase of ROS production by resting PMN from INS patients compared to normal PMN

Savin et al N Engl J Med 1996;334:878-83;Trachtman et al Am J KidneyD is 44: 604–610, 2004 ; McCarthy ET etal J LabClin Med 143:225–229, 2004

Podocytes: Victim or an active player in pathogenesis of NS?







First Hit

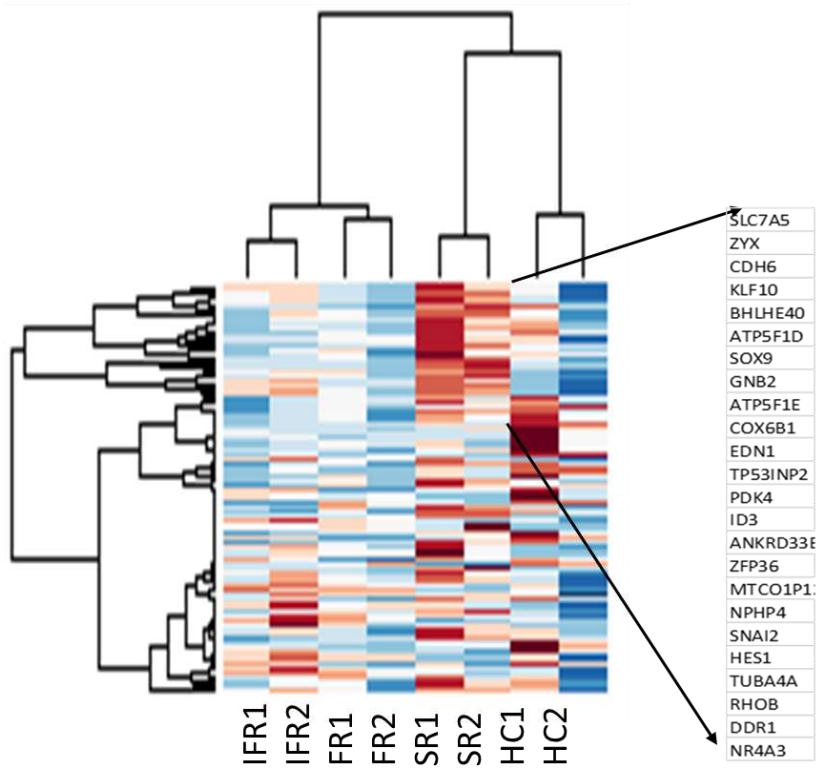
Second Hit
(Impaired CTLA4 response/secretion)

Pediatr Nephrol 2011;**26**:645–9

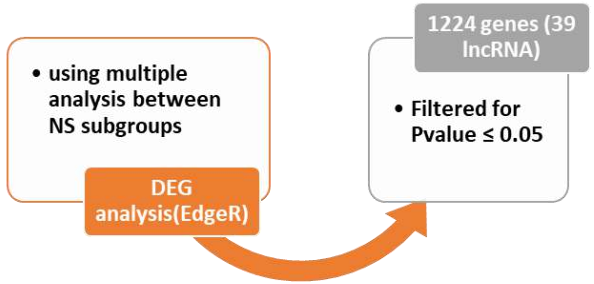
Expression profiling of cultured podocytes exposed to nephrotic plasma reveals intrinsic molecular signatures of nephrotic syndrome

Stuti Panigrahi, MSc[1], Varsha Chhotusing Pardeshi, MSc, PhD[1], Karthikeyan Chandrasekaran, MS[1], Karthik Neelakandan, M. Tech[1], Hari PS, MSc[1], Anil Vasudevan, DNB, MD[1,2]

[1]Division of Molecular Medicine, St. John's Research Institute, St. John's Medical College Bangalore, India
[2]Department of Paediatric Nephology, Institute of Allied Health Sciences, St. John's Medical College, Bengaluru, India



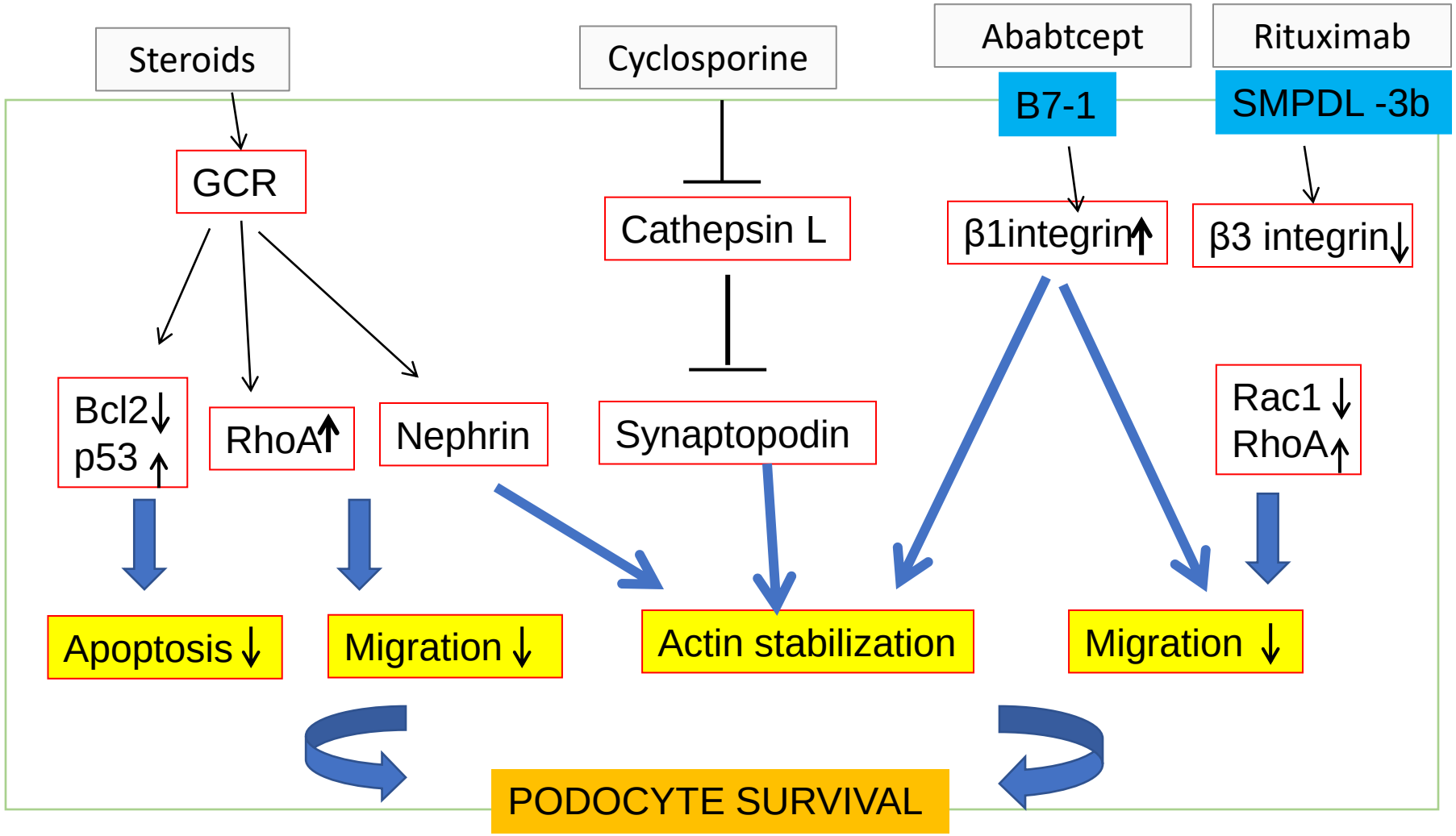
Heatmap of the corresponding normalized values showed clear differences in the expression patterns



Pathway	P Value	Genes
Ribosome	3.40E-07	RPL28,RPL38,RPLP2,RPS15,RPS16,RPL14
NOD-like receptor signaling pathway	8.10E-07	CXCL8,CXCL1,CXCL2,IL6,TNFAIP3
Epithelial cell signaling in Helicobacter pylori infection	0.00048	CXCL8,CXCL1,ATP6V1G1
Chemokine signaling pathway	0.00049	CXCL8,GNB2,CXCL1,CXCL2,CXCL3
Toll-like receptor signaling pathway	0.0022	FOS,CXCL8,IL6
Cytokine-cytokine receptor interaction	0.0028	CXCL8,CXCL1,CXCL2,CXCL3,IL6
MAPK signaling pathway	0.014	FOS,DUSP1,DUSP5,NR4A1
Pathways in cancer	0.1	FOS,CXCL8,IL6

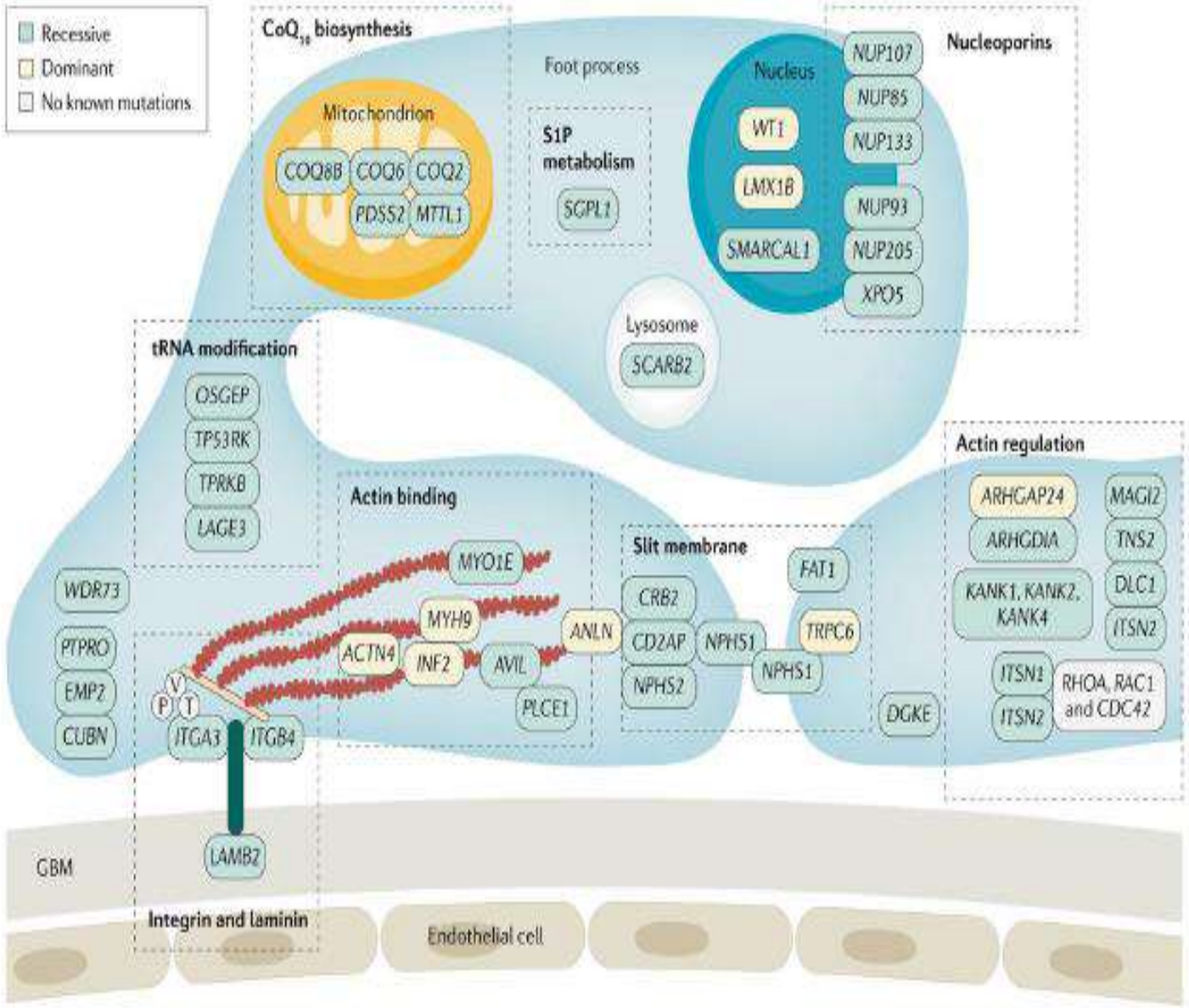
Clin Exp Pediatr (2021)

Immunosuppressive actions on podocytes



Yoo TH et al Kid res Clin Prac 2015;34:69-75

Monogenic causes of nephrotic syndrome



- ~90 known single genes; basis for resistance 10 -30%
- Some genetic variants such as in COQ gene response to medical management

Nat Rev Dis Primers. (2020)

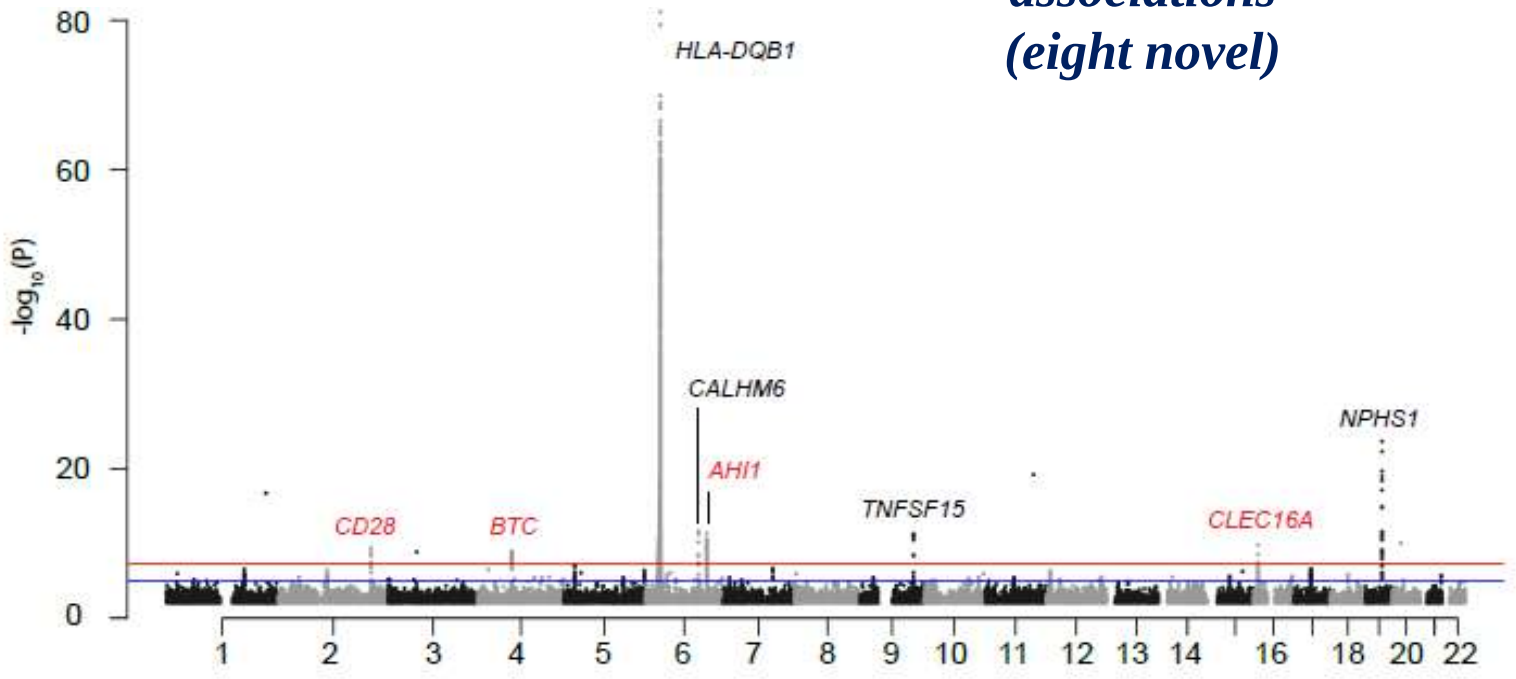
Front Med (Lausanne). 2018 Mar 12;5:55

Genetic susceptibility in nephrotic syndrome

Multi-population genome-wide association study implicates both immune and non-immune factors in the etiology of pediatric steroid sensitive nephrotic syndrome

discovering twelve significant associations (eight novel)

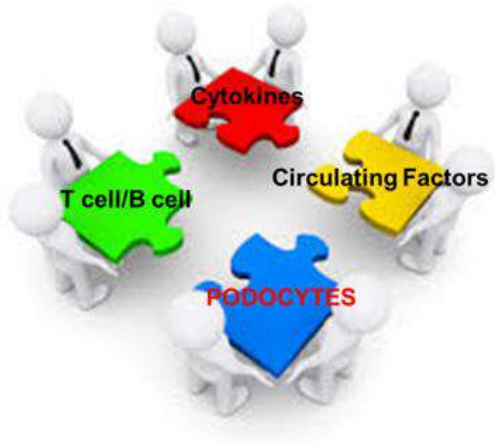
Meta-analysis of 12 GWAS dataset
2,440 cases and 36,023 controls
Admixed American, African, East Asian, European, Maghrebian and South Asian populations



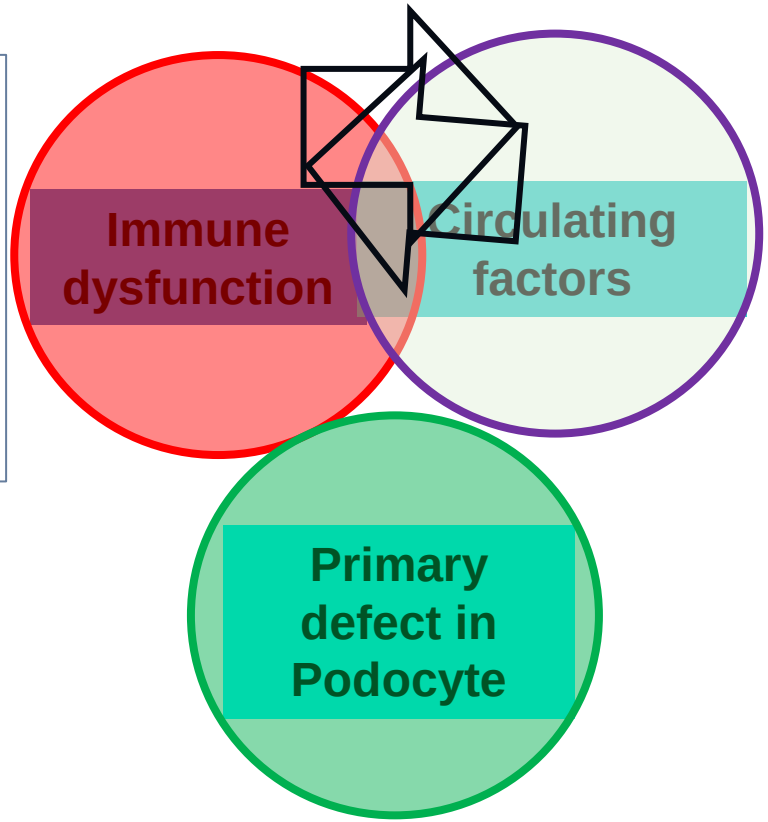
Lead SNPS and closest genes point to immune and kidney biology

medRxiv preprint doi: https://doi.org/10.1101/2022.09.13.22279644

Clinical correlates of pathophysiology of NS



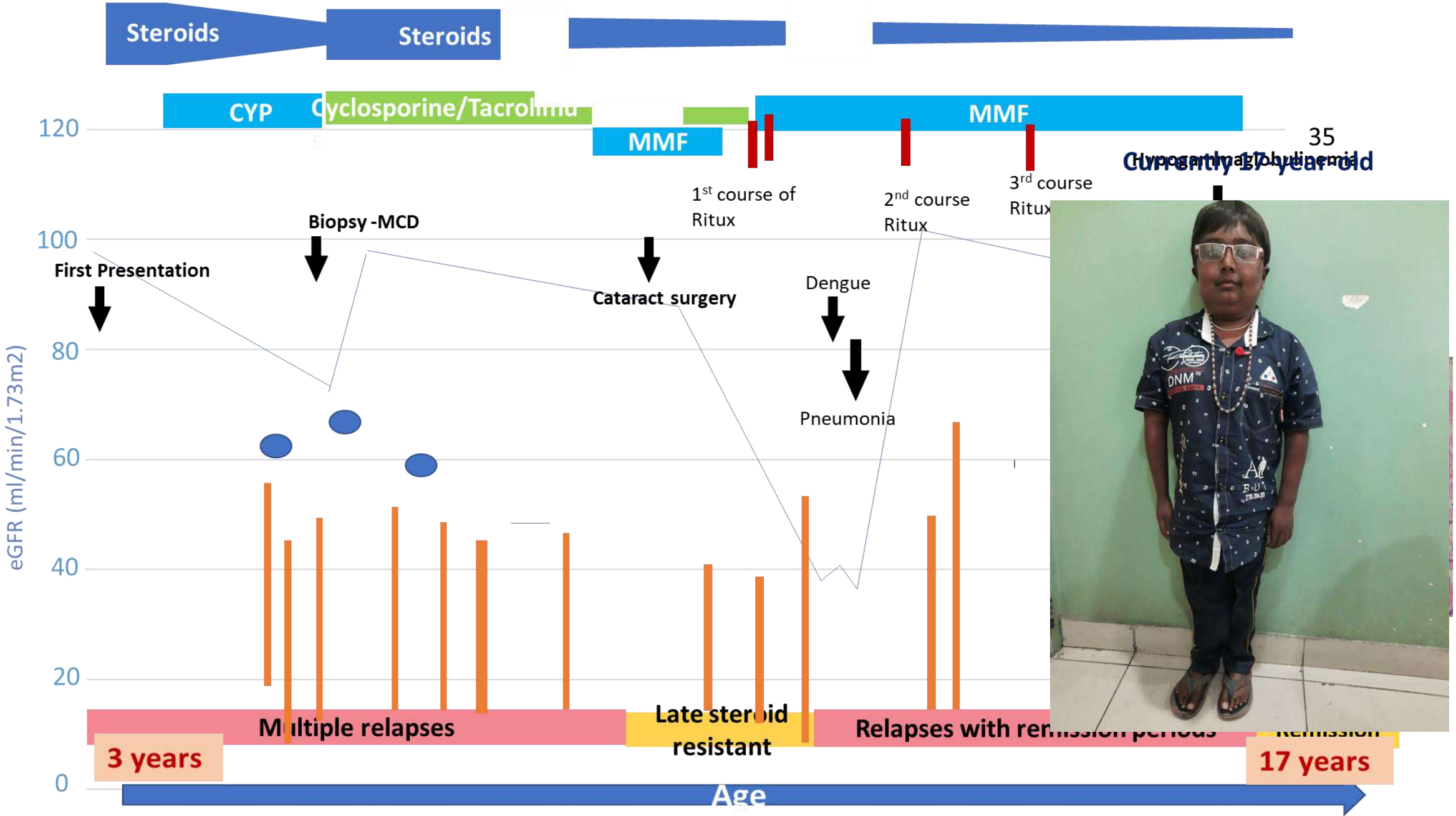
Steroid Sensitive NS
Prompt response to Prednisolone
Steroid sparing agents when in FR/SDNS course
Progression to chronic kidney disease rare



Late SRNS .. Rarely primary SRNS
Variable response to CNI
A proportion progress to end stage kidney disease
Recurrence post transplant

Primary SRNS
Monogenic cause
CNI resistant
Family history of SRNS

Nephrotic Syndrome: An Enigmatic Disease



SUMMARY

- Multiple mechanisms involved in pathogenesis of of idiopathic nephrotic syndrome
- There is a complex interplay between circulating proteins, immune cells and podocytes.
- While monogenic causes associated with SRNS, genetic studies in SSNS indicate a complex genetic basis
- Many aspects related to pathogenesis remain poorly defined
- There is no unitary mechanism that can fully explain the entire pathophysiological process of idiopathic nephrotic syndrome

Indian Academy of Pediatrics (IAP)

STANDARD TREATMENT GUIDELINES 2022

Nephrotic Syndrome

Lead Author
Arvind Bagga

Co-Authors
Anil Vasudevan, Aditi Sinha

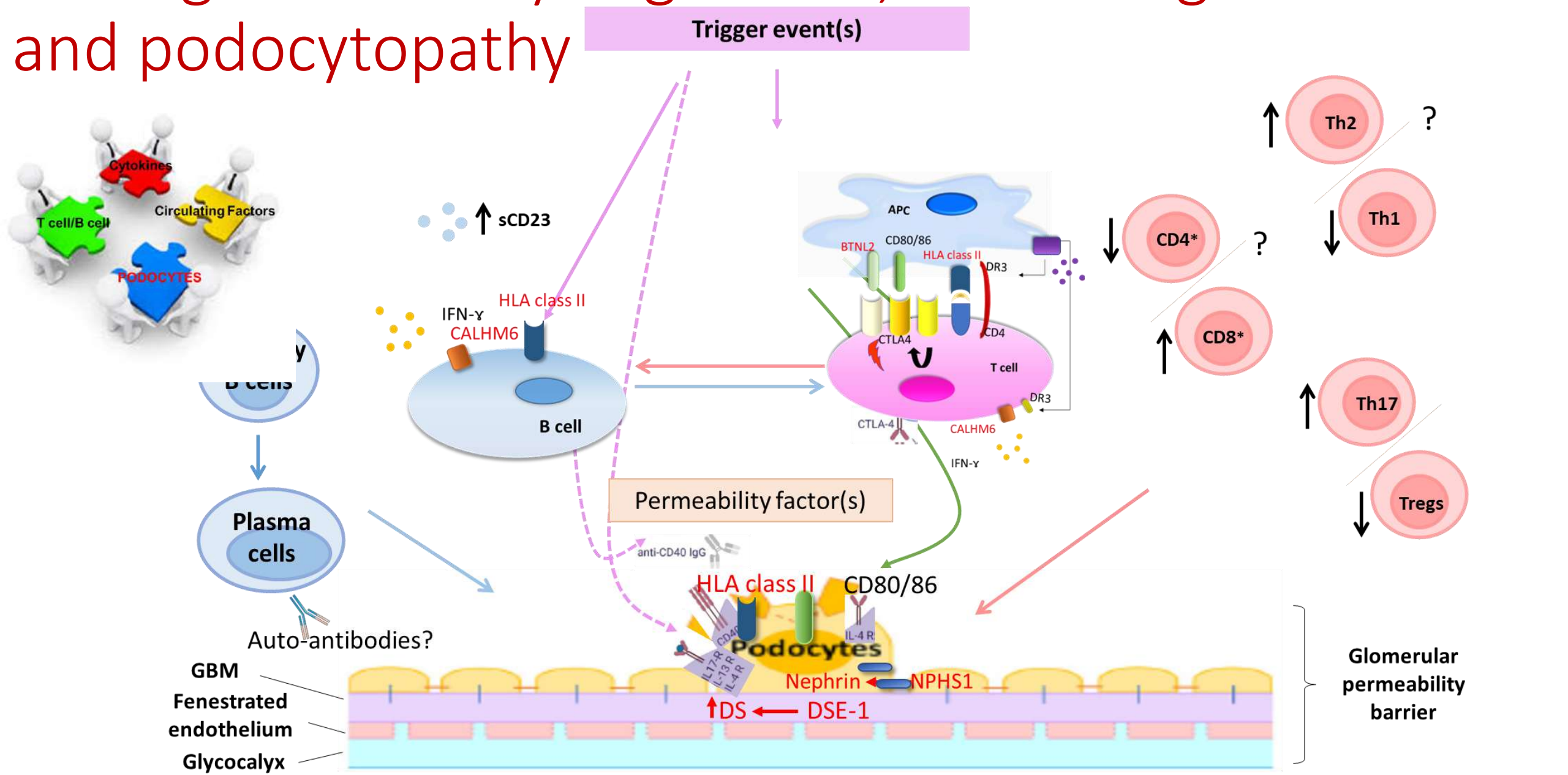
Under the Auspices of the IAP Action Plan 2022

Ramesh Kumar R
IAP President 2022

Piyush Gupta
IAP President 2021

Vinod Saxena
IAP HSC 2022–2025

Linking Immune dysregulation, circulating factors and podocytopathy



Modified Front. Pediatr Nephrol (2018) 33:573–584

Pediatr Nephrol
DOI 10.1007/s00467-012-2163-3

ORIGINAL ARTICLE

TNFα pathway blockade ameliorates toxic effects of FSGS plasma on podocyte cytoskeleton and β3 integrin activation

Martin Bitzan · Sima Babayeva · Anil Vasudevan · Paul Goodyer · Elena Torban

Podocytes exposed to healthy plasma

Podocytes exposed to **SRNS** plasma

Podocytes pre treated with anti TNFα antibody and exposed to **SRNS** plasma