DRAGoN: Deciphering Diversities: Renal Asian Genetics Network

Mutation spectrum among Asian pediatric patients with glomerulopathy

Jun Li Ng[1], Isaac Desheng Liu[1], Mya Than[1], Sadaf Asim[2], Iftikhar Ijaz[3], Duc Quang Nguyen[4], Thi Minh Tan Nguyen[5], Yok ChinYap[6], Caroline Eng[7], Syed Saimul Huque[8], Indra Ganesan[9], Puay Hoon Tan[10], Alwin Loh[10], Sonia Davila[11], Beata S Lipska-Zietkiewicz[12], Franz Schaefer[13], Hui Kim Yap[1], Kar Hui Ng[1]

[1]Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore; Khoo Teck Puat-National University Children's Medical Institute, National University Health System, Singapore; [2]Department of Paediatric Nephrology, National Institute of Child Health, Karachi, Pakistan; [3]King Edward Medical University, Lahore, Pakistan; [4]Children's Hospital 1, Ho Chi Minh City, Vietnam; [5]Children Hospital 2, Ho Chi Minh City, Vietnam; [6]Hospital Kuala Lumpur, Malaysia; [7]Hospital Tuanku Jaafar Seremban, Seremban, Malaysia; [8]Department of Paediatric Nephrology, Bangabandhu Sheikh Mujib Medical University, Dhaka – Bangladesh; [9]KK Women's and Children's Hospital, Singapore; [10]Department of Anatomical Pathology, Singapore General Hospital, Singapore; [11]Institute of Precision Medicine (PRISM), Duke-NUS and Singapore Health Services, Singapore; [12]Clinical Genetics Unit, Medical University Of Gdansk, Gdansk, Poland; [13] Division of Paediatrics Nephrology and KFH Children's Kidney Center, Center for Paediatrics and Adolescent Medicine, Heidelberg, Germany

Introduction

Genetics of glomerulopathies is understudied in many parts of Asia. We set up DRAGoN (Deciphering Diversities: Renal Asian Genetics Network) to study the genetics of primary glomerular diseases in Asia.

Methods

We recruited 163 primary glomerulopathy patients from Singapore (n=72), Vietnam (n=41), Pakistan (n=30), Malaysia (n=19) and Bangladesh (n=1) with disease onset before 2 years; or onset before 25 years with one of following: initial/late steroid-resistance, positive family history (including consanguinity) or extrarenal manifestations. Clinical data were entered into web-based databases every six months. Targeted gene sequencing was performed in 59 genes known to cause glomerulopathy using SeqCap EZ Choice, NimbleGen Roche.

The pathogenicity of the identified variants was evaluated based on the American College of Medical Genetics and Genomics guidelines for clinical sequence interpretation. Only variants in coding exons and canonical splice sites (except for WT1 gene) were considered. Missense mutations which are synonymous or with minor allele frequencies >0.01 for dominant genes or >0.05 for recessive genes were excluded. Missense variants were screened using 6 bioinformatic predictors (SIFT, PolyPhen, MutationTaster, Mutation Assessor, FATHMM and PROVEAN) and CADD. Variants are considered pathogenic if they are truncating, frameshift, essential splice, previously reported mutations, or missense variants deemed deleterious in at least 4 out of 6 *in silico* algorithms and have CADD scores of ≥15.

Results

Median age at first presentation was 3.9 years (range 0.02-24.8). 42 (26%) cases were familial, of which 24 were consanguineous. Median follow-up duration was 4.5 years (range 0.01-39.9) from first presentation. 42 (26%) and 20 (12%) patients had initial and late steroid resistance respectively. 27(17%) responded to calcineurin inhibitors (CNI) while 33 (20%) had resistance. 15(9.2%) patients developed ESRD.

Genetic diagnosis was made in 25 (15.3%) patients. Breakdown according to age at first presentation was: 0-3mths, 30%; 4-12mths, 18%; 1-3yrs, 6.5%; 3-7 yrs, 6.9% and 7-15yrs, 33% (Fig 1). 10 (24%) of familial and 14 (11%) of sporadic cases had genetic diagnosis respectively. Genes involved were *COL4A5* (10), *NPHS1* (5), *NPHS2* (3), *WT1* (4), *TRPC6* (1) and *ADCK4* (2) (Fig 2). *WT1* and *NPHS1* were the commonest implicated genes in congenital/infantile onset, compared to *COL4A5* in those first presenting at 7-15years. 70% of patients with COL4A5 variants first presented at >7 years. Two patients with *COL4A5* or *NPHS2* variants responded completely to CNI (follow-up 0.5-1.4 years).

Figure 1. Percentages of probands with mutations according to ages of first presentation.

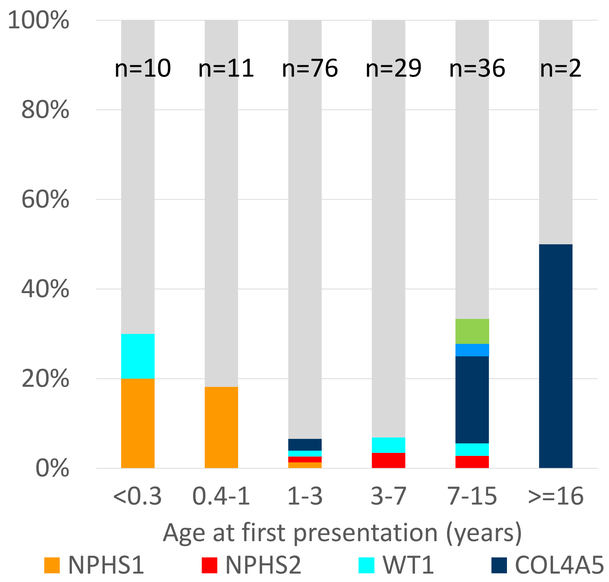
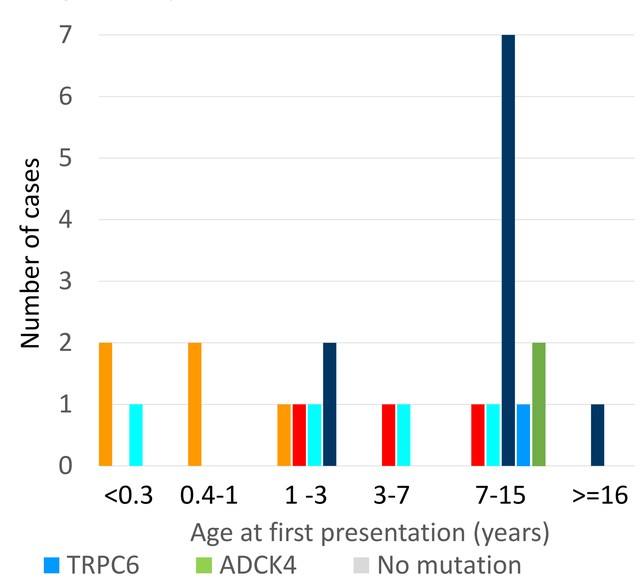


Figure 2. Distribution of implicated genes according to ages of first presentation.



Conclusions

COL4A5 is the commonest gene involved in Asians with genetic glomerulopathy. The higher mutational rate in Asians first presenting >7 years may be related to COL4A5 variants.

Table 1. Details of probands with disease-causing genetic variants.

No.	GENOTYPE					PHENOTYPE												
	Gene / Inheri-tance	GenBank cDNA reference sequence	Type of mutation / Zygosity	Nucleotide change / Amino acid change	Reference SNP cluster ID	Country Ethnicity Gender	First presentation			Histo-logy	Extrarenal manifes-tations	Family history	Treatment Response		Last clinical encounter		
							Age (yrs)	Proteinuria / Serum albumin (g/L)	Clinical presentation				Response to steroids	Response to CNI	Age (yrs)	eGFR (ml/min/ 1.73m²) or CKD Stage	Nephrotic Status
1	**NPHS1** AR	NM_004646	Missense/Hom	c.G2903T/p.G968V	-	PK/ Pakhtoon/F	0.6	Nephotic-range <25	Edema	FSGS	No	Parents are first cousins	Initially resistant	Resistant	1	Rapid eGFR decline from 104 to 55	Subnephrotic proteinuria / normo-albuminemia
2	**NPHS1** AR	NM_004646	Nonsense/Hom	c.C3478T/p.R1160X	rs267606919	PK/Punjabi/M	3.1	Nephotic-range <25	Edema	MCD	No	Consanguinity	Not given	Not given	17	CKD stage 4	Subnephrotic proteinuria
3	**NPHS1** AR	NM_004646	Missense/Het	c.G2206A/p.V736M	rs1131692245	SG/Chinese/M	0.2	Nephotic-range <25	Edema	MCD	Unknown	Unknown	Not given	Not given	10	eGFR 150	No proteinuria
			Missense/Het	c.G928A/p.D310N	rs763972372												
4	**NPHS1** AR	NM_004646	Missense/Het	c.C2491T/p.R831C	rs386833915	MY/Others/F	0.6	Nephotic-range <25	Edema	MCD	No	No	Initially resistant	Not given	2	CKD stage 3	Nephrotic proteinuria / hypo-albuminemia
			Missense/Het	c.T1919G/p.L640R	rs369064893												
5	**NPHS1** AR	NM_004646	Nonsense/Het	c.C2152T/p.Q718X	-	MY/Malay/F	0.02	Nephotic-range <25	Edema	FSGS	No	Brother: NS and epilepsy	Not given	Not given	5	Rapid eGFR decline from 118 to 5	Nephrotic proteinuria / hypo-albuminemia
			Missense/Het	c.C1099T/p.R367C	rs386833865												
6	**NPHS2** AR	NM_014625	FS deletion/Het	c.467delT/p.L156fs	rs758330661	VN/ ? /M	5.1	Nephotic-range <25	Edema	FSGS	No	No	Initially resistant	Resistant	9	Stable eGFR 122	Nephrotic proteinuria
			Missense/Het	c.C524G/ p.P175R	-												
7	**NPHS2** AR	NM_014625	FS deletion/Het	c.293_294del/p.L98fs	-	VN/ ? /F	9.8	Nephotic-range <25	Edema	FSGS	No	No	Unknown	Responsive	11	Stable CKD Stage 3	No proteinuria
			Missense/Het	c.G799T/p.D267Y	-												
8	**NPHS2** AR	NM_014625	Missense/Het	c.C890T/p.A297V	rs199506378	SG/Indian/M	2.8	Subnephrotic 25	Edema	FSGS	No	Sister: FSGS and ESRD	Resistant	Resistant	17	Dialysis at 14 yrs	NA
			Missense/Het	c.G686A/p.R229Q	rs61747728												
9	**WT1** AD		Splice site/Het	IVS9+5G>A	-	MY/Malay/F	13.0	Nephotic-range >30	Renal failure Hypertension	FSGS	Male pseudoherma phroditism Streak gonad	No	Not given	Not given	14	eGFR 19 at presentation, dialysis at 14yrs	Nephrotic proteinuria/ Normo-albuminemia
10	**WT1** AD	NM_000378	Missense/Het	c.C1333T/p.R445W	rs121907900	SG/Indian/F	1.4	Nephotic-range >30	Renal failure Hypertension	FSGS	VUR, previous UTI	No	Not given	Not given	3.5	Dialysis at 2.1 yrs	NA
11	**WT1** AD	NM_000378	Missense/Het	c.C1333T/p.R445W	rs121907900	IDS/ ? /M	3.4	Nephotic-range >30	Hypertension	FSGS	Ambiguous genitalia	No	Initially resistant	Not given	6	eGFR decline from 52 to 20	Subnephrotic proteinuria
12	**WT1** AD	NM_000378	Missense/Het	c.G1250A/p.R417H	rs121907901	SG /Chinese/F	0.02	Nephotic-range <25	Edema Renal failure	-	Streak gonads, 46XX	No	Not given	Not given	3	Dialysis at 3 weeks old	NA
13	**COL4A5** XL	NM_000495	FS deletion/Hem	c.4731_4734del p.H1577fs	.	SG/Chinese/M	1.7	Unknown	Unknown	-	Sensorineural hearing loss	See *	Not given	Partially responsive	19	Last GFR 66	Subnephrotic proteinuria / normo-albuminemia
14	**COL4A5** XL	NM_000495	Missense/Hem	c.G3668A/p.G1223D	-	SG/ ? / M	7.2	Nephotic-range >30	Unknown	Alport	-	No	Not given	Not given	13	CKD stage 3	Nephrotic-range proteinuria
15	**COL4A5** XL	NM_000495	Missense/Hem	c.G3427A/p.G1143S	rs104886228	SG/Chinese/M	16.0	Nephotic-range	Unknown	FSGS	No	2 brothers: dialysis	Not given	Not given	54	CKD stage 3	NA
16	**COL4A5** XL	NM_000495	FS deletion/Hem	c.1797delG/p.K599fs	-	VN/Kinh/M	7.0	Subnephrotic <25	Edema	IgAN	No	Maternal uncle and grandma: renal failure	Not given	Not given	7	Normal	Nephrotic proteinuria / hypo-albuminemia
17	**COL4A5** XL	NM_000495	Truncating/Hem	c.G1210T/p.E404X	.	VN/Kinh/M	12.6	Nephotic-range <25	Edema	MCD	No	No	Initially resistant	Responsive	13	Normal	No proteinuria
18	**COL4A5** XL	NM_000495	FS deletion/Hem	c.4731_4734del/p.H1577fs		VN/ ? /M	9.1	Nephotic-range <25	Edema	FSGS	No	Brother: NS	Initially resistant	Resistant	10	CKD stage 3 eGFR decline 65 to 44	Nephrotic proteinuria
19	**COL4A5** XL	NM_000495	Missense/Hem	c.G2695T/p.G899C	-	VN/ ? /M	12.8	Nephotic-range	Hypertension	FSGS	No	Brother: heavy proteinuria	Not given	Not given	15	CKD stage 1	No proteinuria
20	**COL4A5** XL	NM_000495	Missense/Het	c.G983A/p.G328D	-	SG/Chinese/F	7.8	Subnephrotic	Unknown	MCD	No	Paternal aunt: ESRD	Not given	Not given	?	Unknown	Unknown
21	**COL4A5** XL	NM_000495	Missense/Het	c.G3508A/p.G1170S	rs104886237	IDS/Chinese/F	28.1	Nephotic-range	Hypertension	-	No	See #	Not given	Not given	34	Dialysis at 32yrs, transplant at 33yrs	NA
22	**COL4A5** XL	NM_000495	Missense/Het	c.C3202A/p.P1068T	rs1388513436	VN/Kinh/F	1.9	Nephotic-range <25	Edema	FSGS	No	No	Responsive	Resistant	3	No	Subnephrotic proteinuria
23	**TRPC6** AD	NM_004621	Missense/Het	c.G2689A/p.E897K	rs121434395	SG/Chinese/M	7.8	Nephotic-range <25	Edema Hypertension CKD	FSGS	No	Mum: proteinuria	Initially resistant	Not given (Resistant to Rituximab)	8	Dialysis at 9 yrs	NA
24	**ADCK4** AR	NM_024876	Missense/Het	c.G449A/p.R150Q	rs757644020	SG/Chinese/F	19.3	Nephotic-range >30	Incidental proteinuria	FSGS	No	Sister: ESRD	Resistant	Resistant	26	Dialysis at 22yrs	NA
			Missense/Het	c.C146T/p.R490C	rs750037594												
25	**ADCK4** AR	NM_024876	Splice site/Het	c.289+1G>T	-	IDS/Chinese/F	12.1	Nephotic-range <25	Edema Hypertension Renal failure	Global glomerul osclerosis	No	Brother: Steroid-resistant NS and CKD	Unknown	Not given	20	ESRD and transplant at 12 yrs	No transplant recurrence
			Missense/Het	c.G748C/p.D250H	rs769834604												

Abbreviations: AD, autosomal dominant; AR autosomal recessive; CNI, calcineurin inhibitor; F, Female; FSGS, focal segmental glomerulosclerosis; FS, frameshift; Hem, hemizygous; Het, heterozygous; Hom, homozygous; IDS, Indonesia; IgAN, IgA nephropathy; M, Male; MCD, minimal change disease; MY, Malaysia; NA, not applicable; NS, nephrotic syndrome; PK, Pakistan; SG, Singapore; VN, Vietnam; XL, X-linked

* Mother & maternal aunt: proteinuria & hematuria. Maternal uncle: nephropathy + deafness. Maternal male cousin: ESRD

Mother and 2 sisters: proteinuria & hematuria. Brother: hematuria