

Supplemental Material

Supplemental Methods

Supplemental Tables

Supplemental Table 1: Genotype-phenotype correlation in males with digenic Alport syndrome including a *COL4A5* variant

Supplemental Table 2: Genotype-phenotype correlation in females with digenic Alport syndrome including a *COL4A5* variant

Supplemental Table 3: Genotype-phenotype correlation in males and females with digenic Alport syndrome with a *COL4A3* and *COL4A4* variant

Supplemental Methods

Individuals provided informed consent to the use of their deidentified clinical data at the time of testing or their data in publications or databases were considered to be in the public domain.

Kaplan-Meier plots of kidney survival were constructed as follows.

Males with X-linked Alport syndrome and a pathogenic *COL4A5* variant were identified from LOVD (<https://databases.lovd.nl/shared/genes/COL4A5>) and age at kidney failure extracted. Age at kidney failure was the age at the diagnosis of kidney failure, or where this was not available, the commencement of dialysis, or at first kidney transplant. Affected male family members were included separately. Where only median or mean age at kidney failure was reported for a family, this was used instead. Where a male had not yet progressed to kidney failure, his age at most recent examination was included as a censored data point. Kidney survival curves were compared using the log-rank test.

Survival analysis was performed in R (version 3.6.2) using the *survival* and *survminer* packages ^{1,2,3}.

References

1. Therneau T: A Package for survival analysis in R. R package version 3.2-11. <https://CRAN.R-project.org/package=survival>. 2021
2. Therneau TM, Grambsch PM: *Modeling survival data: Extending the Cox model*, New York, Springer, 2000
3. Kassambara A, Kosinski M, Biecek P: *survminer: Drawing survival curves using 'ggplot2'*. R package version 0.4.9. <https://CRAN.R-project.org/package=survminer>. 2021

Supplemental Table 1: Genotype-phenotype correlation in males with digenic Alport syndrome including a *COL4A5* variant

Ref	Person ID	M/F age	COL4A5 variant	2 nd variant COL4A3	2 nd variant COL4A4	Haematuria	Proteinuria	eGFR now	Age at KF	GBM	Hearing loss	Eye features
Li Zhang 2020	P13	M, 5	c.448G>C, p.Gly150Arg	c.4793T>G, p.Leu1598Arg		YES	?	?	Not yet	Atypical GBM	NO	NO
Zhang 2019	1	M, 11	c.3328_3329 insCAAACCCAG, p.Gly1110Alafs*45	c.4207G>A, p.Gly1403Arg		YES	24hr = 3.08 g/day	133	Not yet	Thinning, thickened, splitting, basket weave	YES (from 6 yrs)	?
Zhang 2019	2	M, 14	Exon42del	c.3946G>A, p.Gly1316Ser		YES	24hr = 3.65 g/day	190	Not yet	?	YES (from 14 yrs)	?
Zhang 2019	3	M, 16	c.1780G>C, p.Gly594Arg		c.4915G>C, p.Gly1639Arg	YES	24hr = 1.63 g/day	145	Not yet	?	?	?
Marina Aksenova	IM	M, 16	c.1871G>A, p.Gly624Asp	c.1219G>C, p.Gly407Arg		YES	0.205 g/m2/day	76	Not yet	AS (14 yrs)	NO	?
Marina Aksenova	KG	M, 18	c.2605G>A, p.Gly869Arg	c.1992delT, p.Gly665Alafs*82		YES	0.755 g/m2/day	70	Not yet	FSGS, AS (15 yrs)	1 gr	?
Marina Aksenova	TD	M, 17	c.3293G>A, p.Gly1098Asp		c.2996G>A, p.Gly999Glu	YES	0.52 g/m2/day	112	Not yet	AS (14 yrs)	NO	?
Marina Aksenova	ShC	M, 17	c.(546+1_610- 1)_(780+1_781-1)del	c.838G>A, p.Gly280Arg		YES	1.082 g/m2/day	85	Not yet	AS (13 yrs)	1 gr	?
Domingo-Gallego 2021	P228	M, ?	c.1643G>A, p.Gly548Asp	c.4649T>G, p.Val1550Gly		YES	YES	?	29	FSGS	?	?
Domingo-Gallego 2021	P230	M, ?	c.465+2T>G	c.2390C>T, p.Pro797Leu		?	?	?	23	Renal cysts	YES	Myopia, astigmatism, strabismus
Mencarelli 2015	Family 10 (II:1)	M, 26	c.4042G>A, p.Gly1348Arg		c.2164G>A, p.Gly722Ser	YES	YES	?	Not yet	?	NO	NO
Mencarelli 2015	Family 11 (I:2)	M, 33	c.2051G>T, p.Gly684Val		c.4760C>G, p.Pro1587Arg	?	?	?	25	?	YES	YES
Mira Choi 2019	Ild	M, 55	c.1871G>A, p.Gly624Asp	c.4484A>G p.Gln1495Arg		YES	YES (from 10 yrs)	?	47	Global foot process effacement	YES	YES
Suman Sethi 2019	Proband	M, 24	5' splice mutation in intron 10 (affecting GT donor site)	indel in ex45 leading to a stop codon at codon 1331		YES	7.8 mg/dL	?	24	?	YES	Lenticonus

Supplemental Table 2: Genotype-phenotype correlation in females with digenic Alport syndrome including a *COL4A5* variant

Ref	Person ID	M/F age	COL4A5 variant	2 nd variant COL4A3	2 nd variant COL4A4	Haematuria	Proteinuria	eGFR now	Age at KF	GBM	Hearing loss	Eye features
Fallerini 2017	Family 3 (II:1)	F, 9	c.2605G>A, p.Gly869Arg	c.3829G>A, p.Gly1277Ser		YES	YES (from 8 yrs)	?	Not yet	Thinning, splitting	NO	NO
Zhao 2019	IID-27	F, 37	c.1339+3A>T		c.4421C>T, p.Thr1474Met	YES	U-P/Cr (g/g) = 1.12	79	Not yet	basket weave	NO	NO
Mastrangelo 2020	Group 2/12	F, 29	c.4793_4798delinsTT, p.Ser1598Phefs*2	c.2765G>T, p.Gly922Glu		YES	Onset uPr/uCr (mg/mg) = 0.4 (age 24 yrs) (peak uPr/uCr = 0.8, age 27 yrs) (current uPr/uCr = 0.17, age 29 yrs)	120	Not yet	?	NO	NO
Mastrangelo 2020	Group 2/13	F, 42	c.2015G>A.p.Gly672Asp	c.5012_5013delGinsTT, *1671Pheext*2		YES	Onset uPr/uCr (mg/mg) = 1.2 (age 22 yrs) (peak uPr/uCr = 5.6, age unknown)	8	40	?	YES (from 18 yrs)	NO
Zhang 2019	4	F, 3	c.50delT, p.Leu17Argfs*27	c.4664C>T, p.Ala1555Val	c.2932G>A, p.Gly978Arg	YES	uPr/uCr (g/g) = 0.32	?	Not yet	Thinning and splitting	?	?
Zhang 2019	5	F, 4	c.3094_3095delAT, p.Met1032Glyfs*35	c.2549G>A, p.Gly850Glu		YES	24hr urinary protein (g/d) = 0.12	?	Not yet	?	?	?
Zhang 2019	6	F, 8	c.4511-2A>G		c.3826G>A, p.Gly1276Arg	YES	24hr urinary protein (g/d) = 0.207	?	Not yet	Thinning, thickening	?	?
Marina Aksenova	AF	F, 9	c.1624G>A, p.Gly542Arg	c.1216C>T, p.Arg406*		YES	0.25 g/m2/day	120	Not yet	?	NO	?
Marina Aksenova	GA	F, 3	c.680G>C, p.Gly227Ala	c.4484A>G, p.Gln1495Arg		YES	NO (0.017 g/m2/day)	108	Not yet	?	NO	?
Marina Aksenova	KV	F, 6	c.4291G>A, p.Asp1431Asn	c.4523A>G, p.Asn1508Ser		YES	0.115 g/m2/day	110	Not yet	?	NO	?
Mencarelli 2015	Family 8 (I:1)	F, 54	c.2858G>T, p.Gly953Val		c.3817+1G>T	?	?	?	44	?	YES	YES
Mencarelli 2015	Family 9 (II:1)	F, 45	c.1931G>A, p.Gly644Asp		c.2075G>T, p.Gly692Val	YES	YES	?	Not yet	?	YES (from 34 yrs)	?
Mencarelli 2015	Family 11 (II:4)	F, 13	c.2051G>T, p.Gly684Val		c.1623+5G>T; c.4760C>G, p.?.; p.(Pro1587Arg)	YES	YES	?	Not yet	?	NO	?

Mencarelli 2015	Family 11 (II:3)	F, 14	c.2051G>T, p.Gly684Val		c.1623+5G>T	YES	YES	?	Not yet	?	NO	?
Mencarelli 2015	Family 11 (II:5)	F, 9	c.2051G>T, p.Gly684Val		c.1623+5G>T	YES	YES	?	Not yet	?	NO	?
Mencarelli 2015	Family 11 (II:6)	F, 5	c.2051G>T, p.Gly684Val		c.1623+5G>T	YES	?	?	Not yet	?	NO	?
Yokota 2017	Case 2	F, 11	c.2732G>A, p.Gly911Glu	c.3691G>A, p.Gly1231Ser		YES	uPr/uCr (g/g) = 1.0 (@11 yrs)	Normal		Thinning, basket weave	?	?
Daga 2018	1108/17 ats	F, 55	c.4689-30_4689- 11		c.592C>T, p.Pro198Ser	YES	NO	?	Not yet	?	NO	NO
Mira Choi 2019	Ila	F, ?	c.1871G>A, p.Gly624Asp	c.4484A>G, p.Gln1495Arg		YES	1.5 g/g creatinine	?	Not yet	?	NO	NO
Mira Choi 2019	IIf	F, ?	c.1871G>A, p.Gly624Asp	c.4484A>G, p.Gln1495Arg		YES	NO	?	Not yet	?	NO	NO
Mira Choi 2019	IIIb	F, 37	c.1871G>A, p.Gly624Asp	c.4484A>G, p.Gln1495Arg		YES	NO	?	Not yet	?	NO	NO
Mira Choi 2019	IIIc	F, 30	c.1871G>A, p.Gly624Asp	c.4484A>G, p.Gln1495Arg		YES	YES	?	Not yet	?	NO	NO
Mira Choi 2019	IIIg	F, ?	c.1871G>A, p.Gly624Asp	c.4484A>G, p.Gln1495Arg		YES	1.7 g/g creatinine	?	Not yet	FSGS	NO	NO
Kyoung Han 2019	Case 1	F, 11	c.4688G>A, p.Arg1563Gln		c.4817G>A, p.Gly1606Glu	YES	Total protein = 7.1 g/dL	?	Not yet	Thickening	NO	Corneal astigmatism

Supplemental Table 3: Genotype-phenotype correlation in males and females with digenic Alport syndrome with a *COL4A3* and *COL4A4* variant

Ref	ID	M/F age	COL4A3 variant	COL4A4 variant	Proteinuria	eGFR now	Age at kidney failure	GBM	Hearing loss	Eye features
Rungsung, 2020		Girl, 18 mo	c.4145G>T, p.G1382V	c.2956C>T, p.Pro986Ser			1.5			
Fallerini, 2017	Family 1	Proband iii:1	c.3829G>A, p.G1277S	c.931-2A>G	YES		52 years	Basket-weave	NO	NO
Fallerini, 2017		Son IV.1, 28 yrs	c.3829G>A, p.G1277S	c.931-2A>G	NO					
Fallerini, 2017		son IV.3, 21 yrs	c.3829G>A, p.G1277S	c.931-2A>G	NO					
Fallerini, 2017	Family 2, II-2	M, 37 yrs	c.3829G>A, p.G1277S	c.3697G>A, p.Gly1233Ser	YES		34 yrs		NO	NO
Fallerini, 2017		Mother 1.1. F, 66yrs	c.3829G>A, p.G1277S	c.3697G>A, p.Gly1233Ser			? Before 66 yrs		YES, 47 yrs	
Fallerini, 2017	Family 4	Proband , II.1, F 49 yrs	c.4523A>G, p.Asn1508Ser	c.5045G>A	YES		49 yrs	Thinned		
Mencarelli, 2015	Family 1	Proband II-2, M7	c.2746+1G>T	c.1553G>A	NO				NO	NO
Mencarelli, 2015	Family 2	Proband II.1, F 36 yrs	c.898G>A, p.Gly300Arg	c.3452G>C	NO				NO	NO
Mencarelli, 2015	Family 3	Proband 1.2, M55yrs	c.1558G>C, p.Gly520Arg	c.4698delT	YES		55 yrs		YES	NO
Mencarelli, 2015		Sister 1.1, F 60 yrs	c.1558G>C, p.Gly520Arg	c.4698delT	NO		50 yrs		YES	NO
Mencarelli, 2015	Family 4	Proband III.1, F 36 yrs	c.1504+1G>A	c.1293_131del	YES		Not yet		NO	
Mencarelli, 2015	Family 5	Proband ii.1, F 3yrs	c.2065G>A, p.Gly689Arg	c.1459+1G>A	NO		Not yet		NO	NO
Mencarelli, 2015	Family 6	Proband II.1, F 37 yrs	c.4994G>A, p.Cys1665Tyr	c.2906C>G	YES					
Mencarelli, 2015	Family 7	Proband II.1, M45 yrs	del exon 1	c(1-?_192+?del);(=)(delex 1 -4)	?		< 45 years			

Kamiyoshi, 2016	Patient 122	M, 35	c.4217G>A, p.G1406Glu	c.2869G>A	0.45	eGFR =54	Not yet		
Kamiyoshi, 2016	Patient 140	M, 16	c.4217G>A, p.Gly1406Glu	c.2869G>A	0.92	eGFR =80	Not yet		
Furlano 2021	Family 32-19-0527	M, 49	c.345del, p.Pro116Leufs*37	c.1100dup	YES	eGFR =47	Not yet		
Furlano 2021	Family 32-190526	F, 59	c.345del, p.Pro116Leufs*37	c.1100dup	YES	10	59		
Furlano 2021	family 32 - 19-0529	F, 30	c.345del, p.Pro116Leufs*37	c.1100dup, p.Pro369Thrfs*61	NO	eGFR =98	Not yet		
Furlano 2021	Family 115-07-66	F, 58	c.4826G>A, p.Arg1609Gln	c.4351G>A, p.Gly1451Arg	NO	80	Not yet		NO
Furlano 2021	Family 115-15-487	M, 42	c.4826G>A, p.Arg1609Gln	c.4351G>A, p.Gly1451Arg	YES	82	Not yet		
Furlano 2021	Family 115-18-129	F, 37	c.4826G>A, p.Arg1609Gln	c.4351G>A, p.Gly1451Arg	NO	85	Not yet		
Furlano 2021	Family 252-15-527	F, 49	c.2342_2350, p.Thr781_Gly783del	c.5045G>A, p.Arg1682Gln	YES	98	Not yet		NO
Furlano 2021	Family 252-16-717	F, 54	c.2342_2350, p.Thr781_Gly783del	c.5045G>A, p.Arg1682Gln	NO				
Furlano 2021	Family 252-16-265	M, 54	c.2342_2350, p.Thr781_Gly783del	c.5045G>A, p.Arg1682Gln	YES	10	54		NO
Furlano 2021	Family 300-17-072	M, 8	c.3829G>A, p.Gly1277Ser	c.193G>A, p.Gly65Ser	YES	109	Not yet		NO
Furlano 2021	Family 336 - 17-515	F, 43	c.3302G>A, p.Gly1101Glu	c.4449_4450dup, p.Met1484Thrfs*69	YES		Not yet		
Furlano 2021	Family 999	F, 44	del2q36.3	dup2q13	YES	74			NO
Zhao	II-D29	m,5	c.3356G>A, p.Gly1119Asp	c.5026C>T, p.Gln1676Ter	0.376	74		Thinned	
Ars	P194	M, 8 years	c.3829G>A, p.Gly1277Ser	c.193G>A, p.Gly65Ser	YES				
Ars	P205	F, unknown	c.4826G>A, p.Arg1609Gln	c.2908C>T, p.Gln970*		<60		FSGS	