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Suppl Table 1: Applicability of ACMG/AMP criteria for *COL4A3 – COL4A5* variants in population frequency databases [24]

Pathogenic criteria	**Description**	**Applicability to population frequency databases for *COL4A3 – COL4A5***
Very strong evidence of pathogenicity (PVS)		
PVS1	Null variant (nonsense, frameshift, *canonical +/- 1 or 2 splice site, initiation codon, single or multi-exon deletion)	YES
Strong evidence of pathogenicity (PS)		
PS1	Same amino acid change as described previously regardless of nucleotide change	YES
PS2	*De novo* variant in a patient with the disease and no family history	NO
PS3	Well-established *in vitro* or *in vivo* functional studies supporting a damaging effect on the gene or gene product	No (not available for *COL4A3 – COL4A5)*
PS4	Prevalence of variant in affected individuals is significantly increased compared with prevalence in controls	NO
Moderate evidence of pathogenicity (PM)		
PM1	Located in a mutational hotspot and/or critical and well-established functional domain	YES**
PM2	Absent from controls (or low prevalence)	YES
PM3	For AR diseases, detected *in trans* with a pathogenic variant	NO
PM4	Protein length changes due in in-frame deletions/insertions or stop loss variants	YES
PM5	Novel amino acid change in an amino acid residue where a different missense change determined to be pathogenic has been seen before	YES
PM6	Assumed *de novo*, but without confirmation of paternity and maternity	NO
Supporting evidence of pathogenicity (PP)		
PP1	Co-segregation with disease in multiple affected family members in causative gene	NO
PP2	Missense variant where these are a common mechanism of disease	YES
PP3	Multiple lines of computational evidence support a deleterious effect (conservation, evolutionary, splicing impact etc)	YES
PP4	Patient's phenotype or family history is highly specific for a disease with a single genetic aetiology	NO
PP5	Reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory	YES
Benign criteria		

Stand alone evidence of benign impact (BA)		
BA1	Allele frequency is above 5% in EVS, 1000 genomes or ExAC	NO
Strong evidence of benign impact (BS)		
BS1	Allele frequency is greater than expected for disorder	YES
BS2	Observed in a healthy adult individual for X-linked disorder with full penetrance expected at an early age	NO
BS3	Well-established *in vitro* or *in vivo* functional studies shows no damaging effect on protein function or splicing	NO
BS4	Lack of segregation in affected members of a family	NO
Supporting evidence of benign impact (BP)		
BP1	Missense variant in a gene for which primarily truncating variants are known to cause disease	NO
BP2	Observed *in trans* with a pathogenic variant for a fully penetrant dominant gene/disorder, or *in cis* with a pathogenic variant in any inheritance pattern	NO
BP3	In-frame deletions/insertions in a repetitive region without a known function	YES
BP4	Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact etc)	YES
BP5	Variant found in a case with an alternate molecular basis for disease	NO
BP6	Reputable source recently reports variant as benign but the evidence is not available for an independent evaluation	YES
BP7	A synonymous variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved	YES

PVS1, PS1, PM1 etc are explained in the Table

*A recent publication has warned against the use of PP3 for canonical splice-site variants but these are still used by Varsome[36], and we did not alter Varsome assessments.

** https://www.acgs.uk.com/media/11285/uk-practice-guidelines-for-variant-classification-2019-v1-0-3.pdf

Suppl Table 2: Non-collagenous domains and interruptions in collagen IV α5, α3 and α4 chains[28, 26, 27]

Collagen IV α5 chain	Collagen IV α3 chain	Collagen IV α4 chain
Amino -NC domain (1-41)	Amino NC (1-42)	Amino NC (1-61)
I (160-167)	I (160-170)	I (176-183)
II (220-223)	II (222-223)	II (235-236)
III (243-257)	III (245-258)	III (258-269)
IV (282|283)*	IV (283-287)	IV (294-295)
V (344-355)	V (345-353)	V (359-366)
VI (390-393)	VI (387-388)	VI (400-401)
VII (416-419)	VII (413-414)	VII (429-432)
VIII (442-453)	VIII (442-445)	VIII (457-462)
IX (479-487)	IX (476-483)	IX (493-496)
X (549-554)	X (544-547)	X (560-565)
XI (595-599)	XI (587-589)	XI (605-609)
XII (625)	XII (617-618)	XII (631-632)
XIII (657-662)	XIII (649-655)	XIII (666-673)
XIV (706)	XIV (698-699)	XIV (716-718)
XV (753-756)	XV (745-749)	XV (740-741)
XVI (818)	XVI (810-811)	XVI (763-764)
XVII (853-856)	XVII (848-849)	XVII (828-830)
XVIII (954-960)	XVIII (946-951)	XVIII (966-971)
XIX (1070-1073)	XIX (1060-1064)	XIX (1014)
XX (1189)	XX (1176-1179)	XX (1078-1081)
XXI (1245-8)	XXI (1234-1235)	XXI (1196-1197)
XXII (1373-78)	XXII (1263-1264)	XXII (1222-1223)
Carboxy-NC domain (1461-1691)	XXIII (1352-1357)	XXIII (1251-1257)
	Carboxy- NC (1439-1670)	XXIV (1285-1288)
		XXV (1370-1379)
		XXVI (1404-1405)
		Carboxy-NC (1460-1690)

*This is not an interruption but rather is formed from two Gly residues adjacent to each other.

Suppl Table 3: Assessment of *COL4A5* variants in the 100,000 Genomes Project database and correlation with haematuria

Protein consequence	Transcript	PP2	SIFT	M T	Cons	gnomAD (Hem/ Het, total alleles)	Previously reported; or substitution with other residue (LOVD)	ClinVar	Varsome	Predicted pathogenic	Number in 100,000 Genomes Project with haematuria	Number in 100,000 Genomes Project without haematuria
p.(Gly93Cys)	c.277G>T	1	0	1	Gly	None	NO		P (PM1,PM2,PM5,PP2,PP3)	YES	1	0
p.(Gly108Glu)	c.323G>A	1	0	1	Gly	None	NO		P (PM1,PM2,PM5,PP2,PP3)	YES	1	0
p.(Gly138Cys)	c.412G>T	1	0	1	Gly	None	NO; other - LP/P	LP*	LP (PM1,PM2,PM5,PP2, PP3, PP5)	YES	0	1
p.(Gly307Ser)	c.919G>A	1	0	1	Gly	None	YES- LP, other-P		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly325Arg)	c.973G>A	0.999	0	1	Gly	None	YES - P	P*	P (PS1,PM1,PM2,PM5,PP2, PP3,PP5)	YES	1	0
p.(Gly426Arg)	c.1276G>A	1	0.001	1	Gly	None	YES - P		P (PS1,PM1,PM2,PP2,PP3)	YES	1	0
p.(Gly491Arg)	c.1471G>A	0.999	0.003	1	Gly	None	Other - P		LP(PM1,PM2,PM5,PP2,PP3)	YES	0	1
p.(Gly533Arg)	c.1597G>A	1	0	1	Gly	None	Other - P		LP (PM1,PM2,PM5,PP2,PP3)	YES	1	0
p.(Gly635Arg)	c.1903G>C	1	0	1	Gly	None	LP; other -P		P (PM1,PM2,PM5,PP2,PP3)	YES	1	0
[GN] p.(Gly624Asp)	c.1871G>A	1	0.021	1	Gly	16/ 182,998	Slajpah (2007)	P**	P (PM1,PP2,PP3,PP5)	YES	0	1
p.(Gly653Val)	c.1958G>T	1	0	1	Gly	None	Other x2 - P		LP (PM1, PM2, PM5, PP2,PP3)	YES	1	0
p.(Gly693Val)	c.2078G>T	1			Gly	None	Other x2- LP		LP (PM1,PM2, PM5,PP2, PP3)	YES	1	0
p.(Gly707Val)	c.2120G>T	1	0.002	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3	YES	1	0
p.(Gly743Ala)	c.2228G>C	1	0	1	Gly	None	Other - P		LP (PM1,PM2,PM5,PP2,PP3)	YES	0	1

GN p.(Gly752Val)	c.2255G>T	1	0.031	1	Gly	2/183,277	Other – P		LP (PM1,PM2,PP2,PP3)	YES	0	3
p.(Gly953Val)	c.2858G>T	1	0.001	1	Gly	705/204,819	Knebelman (1996)	Conflicting*	VUS (PM1,PP2,PP3,PP5, BS1,BS2)	NO	5	63
p.(Gly1066Ser)	c.3196G>A	1	0	1	Gly	None	Other x 3 - P	P*	P (PS1, PM1,PM2, PM5,PP2,PP3,PP5)	YES	1	0
p.(Gly1388Ser)	c.4162G>A	1	0.005	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly1421Asp)	c.4262G>A	1	0	1	Gly	None	Other - LP		LP (PM1,PM2,PP2,PP3)	YES	1	0
p.(Gly1433Ser)	c.4297G>A	1	0	1	Gly	None	Other -3 in 4 - LP		P (PVS1, PM1, PM2, PM5, PP2, PP3)	YES	1	0
Total number with a predicted pathogenic variant with and without haematuria respectively											12	9
Total number with predicted non-pathogenic variant with or without haematuria respectively											5	63
Total number with haematuria and without haematuria respectively											2221	37,200

GnomAD indicates that the variant is also found in gnomAD database. Data highlighted in blue are those with evidence against pathogenicity, and conclusion and number of individuals from 100,000 Genomes Project with this variant with or without haematuria are. PP2 (Polyphen-2), SIFT (Sorting Intolerant from Tolerant), Mutation taster are computational tools to assess pathogenicity, and scores of >0.80, Del (for deleterious) or DC (disease-causing) are consistent with pathogenicity respectively. Conservation of Gly was examined to vertebrates (in birds). ClinVar – uses P pathogenic, LP Likely pathogenic, VUS – variant of uncertain significance, B – benign, LB likely benign, and star system used for quality of assertion * to **. Varsome uses ACMG/AMP grading of P, LP,VUS,LB and B and criteria of PVS, PM, PP, BS etc. The tools used here have been to principally exclude variants where the evidence is not supportive of pathogenicity.**

Of especial interest was p.(Gly953Val) which was not predicted pathogenic and excluded from the population frequency. It was assessed as pathogenic in only two of the three computational tools, and had a conflicting ClinVar assessment. It was present 691 times in the gnomAD cohort almost always in people of East or South Asian ancestry. Alamut considered it Likely Pathogenic based on 2 moderate (PM1-PM6) and > 4 supporting (PP1-PP5) criteria, but Varsome assessed it as Benign (PM1, PP2, PP3, BS1, BS2). This variant has been described previously as hypomorphic[41], but has also been detected on the same allele as other pathogenic *COL4A5* variants[42, 43]. A recent study with clinical data from several families concluded that it was not pathogenic[44].

Suppl Table 4: Assessment of *COL4A3* variants in the 100,000 Genomes Project database and correlation with haematuria

Protein consequence	Transcript	PP2	SIFT	M T	Cons	gnomAD (Hem/ Het, total alleles)	Previously reported; or substitution with other residue (LOVD)	ClinVar	Varsome	Predicted pathogenic	Number in 100,000 Genomes Project with haematuria	Number in 100,000 Genomes Project without haematuria
p.(Gly46Arg)	c.136G>A	1	0	0.999	Gly	61/ 280,882	LB	LB**	LP (PM1,PM2PP2,PP3,BP6)	NO	0	10
p.(Gly49Arg)	c.145G>C	1	0.14 (Tol)		Gly	None	VUS		VUS (PM1,PM2, PP2)	NO	0	1
p.(Gly49Glu)	c.146G>A	1	0.002	0.9999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly58Arg)	c.172G>C	1	0	0.9999	Gly	1/ 240,996	Other x1 P		LP (PM1,PM2,PM5,PP2,PP3)	YES	0	1
p.(Gly58Asp)	c.173G>A	1	0	0.9999	Gly	None	Other x1 P		LP (PM1,PM2,PM5,PP2,PP3)	YES	0	1
p.(Gly58Ser)	c.172G>A	1	0	0.9999	Gly	6/ 243,384	Other x1 P	VUS*	LP (PM1,PM2,PM5,PP2,PP3)	YES	0	6
p.(Gly67Ter)	c.199G>T				Gly	None	NO		P (PVS1,PM2,PP3)	YES	0	1
p.(Gly70Arg)	c.208G>C	1	0.002	0.9999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
^{GN}p.(Gly94Glu)	c.281G>A	1	0	0.9999	Gly	1/ 249,140	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly94Asp)	c.281G>A	1			Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly97Ser)	c.292G>A	1			Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly97Cys)	c.292G>T	1	0	0.9999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
^{GN}p.(Gly100Arg)	c.298G>A	1	0	0.9999	Gly	1/ 249,204	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly112Ser)	c.334G>A	1	0	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly115Asp)	c.341G>A		0	0.9999	Gly	None	LP	P	LP (PM1,PM2,PP2,PP3,PP5)	YES	0	1
p.(Gly118Arg)	c.352G>A	1	0	0.9121	Gly		NO		LP (PM1,PM2,PP2,PP3)	YES	1	1
^{GN}p.(Gly124Glu)	c.371G>A	1	0	0.9999	Gly	1/ 249,394	NO		LP (PM1,PM2,PP2,PP3)	YES	0	2
p.(Gly139Arg)	c.415G>C	1	0	0.9999	Gly	None	P	VUS**	LP (PM1,PM2,PP2,PP3)	YES	1	1
p.(Gly148Val)	c.443G>T	1	0.002	1	Gly	4/ 280,874	LP, VUS		LP (PM1,PM2,PP2,PP3)	YES	1	1
p.(Gly174Val)	c.521G>T	1	0	1	Gly	None	Other x2 P		LP,PM1,PM2,PM5,PP2,PP3)	YES	1	0

GNp.(Gly183Asp)	c.548G>A	1	0.001	1	Gly	1/ 249,322	Other x 3 P		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly201Glu)	c.602G>A	1	0.002	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	2
p.(Gly204Val)	c.611G>T	1	0.002	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly213Arg)	c.637G>A	1	0.001	0.9999	Gly	None	NO		P (PS1,PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly242Glu)	c.725G>A	1	0.003	0.9999	Gly	None	P	LP*	LP (PM1,PM2,PP2,PP3,PP5)	YES	0	1
p.(Gly291Glu)	c.872G>A	1	0	1	Gly	None	P	LP	LP (PM1,PM2,PP2,PP3,PP5)	YES	0	1
GNp.(Gly300Arg)	c.898G>A	1	0	0.9999	Gly	5/ 249,426	LP, VUS		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly372Ser)	c.1114G>A	1	0.006	0.9998	Gly	None	NO		P (PVS1,PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly378Glu)	c.1133G>A	1	0.000 2	0.9936	Gly	None	LP		LP (PM1,PM2,PP2,PP3)	YES	1	0
GNp.(Gly389Asp)	c.1166G>A	1	0.002	0.9999	Gly	1/ 248,214	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly407Ala)	c.1220G>C	1	0.008	1	Ile	None	Other x 1 VUS		LP (PM1,PM2,PM5,PP2,PP3)	NO	1	0
GNp.(Gly436Ala)	c.1307G>C	1	0.01	0.9999	Gly	5/ 280,838	NO		LP (PM1,PM2,PP2,PP3)	YES	0	2
GNp.(Gly484Arg)	c.1450G>A	1	0.018	0.9999	Gly	7/ 249,506	NO		P (PS1,PM1,PM2,PP2,PP3)	YES	0	3
p.(Gly487Val)	c.1460G>T	1	0.012	0.9999	Gly	None	Other x 1 P		LP (PM1,PM2,PM5,PP2,PP3)	YES	0	1
p.(Gly487Ser)	c.1459G>A	1	0.038 (Dam)	0.7711 (Pol)	Gly	31/ 280,912	Other x 1 P		LP (PM1,PM2,PM5,PP2,PP3)	NO	0	4
p.(Gly511Arg)	c.1531G>A	1	0.001	0.9999	Gly	None	Other x 1 LP		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly511Ala)	c.1532G>C	1	0.003	0.9999	Gly	None	Other x 1 LP		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly532Cys)	c.1594G>T	1	0.002	1	Gly	None	P or VUS; other x1 LP	P, LP**	P (PP5, PM1,PM2,PM5,Pp2,PP3)	YES	0	1
p.(Gly560Val)	c.1679G>T	1	0	0.9999	Gly	None	LP		LP (PM1,PM2,PP2,PP3)	YES	1	0
p.(Gly575Arg)	c.1723G>A	1	0	0.9999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	1	0
p.(Gly575Glu)	c.1724G>A	1	0	0.9999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	1	0
p.(Gly575Val)	c.1724G>T	1	0	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly602Asp)	c.1805G>A	0.795	0.003	0.9999	Gly	None	NO		LP (PM1,PM2, PP2,PP3)	NO	0	1
p.(Gly611Glu)	c.1832G>A	1	0.002	0.9999	Gly	None	VUS		LP (PM1,PM2,PM5,PP2,PP3)	YES	0	1
GN.(Gly619Arg)	c.1855G>A	1	0.002	0.9999	Gly	2/ 246,972	LP, P, or VUS		P (PS1,PM1,PM2,PP2,PP3)	YES	1	0

[GN]p.(Gly637Arg)	c.1909G>A	1	0	0.9999	Gly	2/ 246,114	NO		P (PS1,PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly680AspfsTer70)	c.2031_2038dup	1			Gly	2/ 244,894 P	P		P (PVS1, PM2,PP3,PP5)	YES	0	2
p.(Gly695Glu)	c.2084G>A	1	0	0.9999	Gly	None	Other x 1 LP, P or VUS		LP (PM1,PM2,PM5,PP2,PP3)	YES	0	1
p.(Gly695Arg)	c.2083G>A	1	0	0.9999	Gly	31/ 278,152	LP, P or VUS	Con-flicting	P (PS1, PM1,PM2,PP2,PP3)	NO	3	18
p.(Gly700Glu)	c.2099G>A	1	0.001	0.9999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly721ValfsTer26)	c.2162del	1	0	1	Gly	3/ 243,442	NO	P *	LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly730Glu)	c.2189G>A	1	0	0.9999	Gly	None	NO	VUS	LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly780Glu)	c.2339G>A	1	0	1	Gly	None	LP		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly792Glu)	c.2375G>A	1	0	1	Gly	None	P; other x1 LP		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly801Glu)	c.2402G>A	1	0	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	1	1
p.(Gly807ArgfsTer28)	c.2417dup	1	0.002	0.9999	Gly	1/ 172,936	P	LP**	P (PVS1,PM2,PP3,PP5)	YES	0	2
p.(Gly812Ser)	c.2434G>A	0.339	0.019	0.9986 (Pol)	Gly	10/ 178,112	VUS		VUS (PM1,PM2,PP2,BP4)	NO	0	6
[GN]p.(Gly818Arg)	c.2452G>A	1	0	1	Gly	2/ 174,872	LP, VUS	P	P (PS1,PM1,PM2,PP2,PP3)	YES	3	4
p.(Gly824Glu)	c.2471G>A	1	0	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly856Glu)	c.2567G>A	0.997	0	1	Gly	None	LP, P or VUS		LP (PM1,PM2,PP2,PP3)	YES	1	0
p.(Gly874Ter)	c.2620G>T			1	Gly	None	NO		P (PVS1,PM2,PP3	YES	0	1
p.(Gly874AspfsTer9)	c.2621del	1			Gly	10/ 280,726	NO	LP*	P (PVS1,PM2,PP3,PP5)	YES	1	6
p.(Gly883Arg)	c.2647G>A	1	0	1	Gly	None	NO		P (PS1,PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly895Asp)	c.2684G>A	1	0	1	Gly	None	NO	LP	LP (PM1,PM2,PP2,PP3,PP5)	YES	0	1
p.(Gly943Arg)	c.2827G>A	1	0	0.9999	Gly	2/ 249,332	NO		LP (PM1,PM2,PP2,Pp3)	YES	0	4
p.(Gly1128Ala)	c.3383G>C	0.999	0	1	Gly	None	NO		LP (PM1,PM2,PP2,Pp3)	YES	0	1
p.(Gly1137Ser)	c.3409G>A	1	0	1	Gly	None	Other x 1 P		LP (PM1,PM2,PM5,PP2,PP3)	YES	0	1
p.(Gly1152Val)	c.3455G>T	1	0	1	Gly	None	NO		LP (PM1,PM2,PM5,PP2,PP3)	YES	0	1

p.(Gly1158Arg)	c.3472G>C	1	0	1	Gly	1/ 249,490	VUS	Con-flicting *	LP (PM1,PM2,PP2,PP3)	NO	0	1
GNp.(Gly1167Arg)	c.3499G>A	1	0	1	Gly	2/ 249,476	LP, P, VUS	P,LP	P (PS1,PM1,PM2,PP2,PP3,PP5)	YES	1	3
p.(Gly1198Ser)	c.3592G>A	1	0	1	Gly	None	NO	LP	LP (PM1,PM2,PM5,PP2,PP3,PP5)	YES	1	0
GNp.(Gly1207Arg)	c.3619G>C	1	0.532	0.9981	Gly	1/ 234,658	Other x1, P	P	VUS (PM1PM2,PP2)	NO	1	0
p.(Gly1231Ser)	c.3691G>A	1	0.006	1	Gly	None	Other x1, P	VUS	LP (PM1,PM2,PP2,PP3)	YES	0	3
GNp.(Gly1254Arg)	c.3760G>C	1	0.002	1	Gly	1/ 249,506	P		LP (PM1,PM2,PP2,PP3)	YES	3	1
p.(Gly1268Glu)	c.3803G>A	1	0	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
GNp.(Gly1277Ser)	c.3829G>A	1	0	1	Gly	102/ 280,750	LP, P or VUS	VUS	P (PS1,PM1,PM2,PP2,PP3,PP5)	NO	2	28
p.(Gly1280Cys)	c.3838G>T	1	0	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	1	1
p.(Gly1283Glu)	c.3848G>A	1	0	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly1298Arg)	c.3892G>C	1	0.001	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	1	0
p.(Gly1304Arg)	c.3910G>A	1	0	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	2
p.(Gly1313Glu)	c.3938G>A	1	0	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly1328Val)	c.3983G>T	1	0.002	1	Gly	8/ 280,792	NO		LP (PM1,PM2,PP2,PP3)	YES	0	2
GNp.(Gly1367Arg)	c.4099G>C	1	0	1	Gly	1/ 249,320	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly1376Arg)	c.4126G>C	1	0.001	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
GNp.(Gly1397Glu)	c.4190G>A	1	0.002	1	Gly	1/ 249,248	NO		LP (PM1,PM2,PP2,Pp3)	YES	0	1
GNp.(Gly1400Glu)	c.4199G>A	1	0	1	Gly	2/ 249,256	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly1418Glu)	c.4253G>A	1	0	1	Gly	None	LP		LP (PM1,PM2,PP2,PP3)	YES	1	0
Total number with a predicted pathogenic variant with haematuria or without respectively											21	85
Total number with haematuria or without haematuria respectively											2,221	37,200

Variants with any evidence not consistent with pathogenicity are highlighted in blue. Otherwise notes are the same as for Suppl Table 3.

Suppl Table 5: Assessment of *COL4A4* variants in the 100,000 Genomes Project database and correlation with haematuria

Protein consequence	Transcript	PP2	SIFT	M T	Cons	gnomAD (Hem/ Het, total alleles)	Previously reported; or substitution with other residue (LOVD)	ClinVar	Varsome	Predicted pathogenic	Number in 100,000 Genomes Project with haematuria	Number in 100,000 Genomes Project without haematuria
p.(Gly1459Val)	c.4376G>T	0.627	0.044 (Dam)	0.9994 (Pol)	Gly	2/ 274,180	LP		VUS (PM1,PM2,PP2, BP4)	NO	0	1
GNp.(Gly1430Arg)	c.4288G>A	1	0.001	1	Gly	1/ 249,468	NO	P*	LP (PM1,PM2PP2,PP3,PP5)	YES	0	1
p.(Gly1418Asp)	c.4253G>A	1	0.003	1	Gly	None	NO		LP (PM1,PM2PP2,PP3)	YES	0	1
p.(Gly1401ProfsTer31)	c.4200_4201del	1			Gly	None	P		P (PVS1,PM2,PP3)	YES	0	1
p.(Gly1380Asp)	c.4139G>A	1	0.001	0.999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	2
GNp.(Gly1346Val)	c.4037G>T	1	0.002	1	Gly	4/ 249,206	NO		LP (PM1,PM2,PP2,PP3)	YES	0	2
GNp.(Gly1325Arg)	c.3973G>C	1	0	0.9999	Gly	9/ 249,006	NO		P (PVS1,PM2,PP2,PP3)	YES	0	4
p.(Gly1319Arg)	c.3955G>A	1	0	0.9999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly1295Asp)	c.3884G>A	1	0.002	0.9998	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly1292Asp)	c.3875G>A	1	0	1	Gly	None	LP	LP	LP (PM1,PM2,PP2,PP3,PP5)	YES	1	0
p.(Gly1258Asp)	c.3773G>A	1	0.005	0.998	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	2
GNp.(Gly1248Glu)	c.3743G>A	1	0.003	0.9999	Gly	17/ 280,842	VUS	LP*	LP (PM1,PM2,PP2,PP3,PP5)	YES	0	1
p.(Gly1230Asp)	c.3689G>A	1	0.003	0.9999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
GNp.(Gly1201Asp)	c.3602G>A	1	0	0.9999	Gly	1/ 249,040	LP		LP (PM1,PM2,PM5,PP2,PP3)	YES	0	1
GNp.(Gly1178Ser)	c.3532G>A	1	0	0.9999	Gly	24/ 280,386	LP, VUS		LP (PM1,PM2,PP2,PP3)	YES	0	3

GNp.(Gly1151Ala)	c.3452G>C	1	0	1	Gly	10/249,432	NO		LP (PM1,PM2,PP2.PP3)	YES	0	7
GNp.(Gly1136Ala)	c.3407G>C	1	0	0.9999	Gly	3/280,616	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly1124Val)	c.3371G>T	1	0.001	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
GNp.(Gly1103Arg)	c.3307G>A	1	0.001	0.9999	Gly	5/249,156	P, VUS	LP*	LP (PM1,PM2,PP2,PP3,PP5)	YES	0	2
p.(Gly1082Val)	c.3245G>T	1	0.002	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly1069SerfsTer4)	c.3204_3205insTCTT	1			Gly	None	NO		P (PVS1,PM2,PP3)	YES	2	0
p.(Gly1018Arg)	c.3052G>C	1	0	1	Gly	4/249,580	NO		LP (PM1,PM2,PP2,PP3)	YES	0	2
p.(Gly1015Arg)	c.3043G>A	1	0.001	1	Gly	None	Other x1 LP		LP (PM1,PM2,PM5,PP2,PP3)	YES	0	1
p.(Gly1015Glu)	c.3044G>A	1	0.002	1	Gly	14/280,944	Other x1 LP	Con-flicting*	LP ((PM1,PM2,PP2,PP3,PP5)	NO	1	8
GNp.(Gly996Arg)	c.2986G>A	1	0	1	Gly	10/280,858	P, VUS	P*	LP (PM1,PM2,PP2,PP3,PP5)	YES	0	4
GN p.(Gly990Asp)	c.2969G>A	1	0	1	Gly	4/249,330	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
GN p.(Gly963Glu)	c.2888G>A	0.997	0.272 (Tol)	0.9999	Gly	2/249,566	NO		LP (PM1.PM2,PP2,PP3)	NO	1	0
GN p.(Gly960Arg)	c.2878G>A	1	0.002	1	Gly	3/249,540	NO	LP or P **	P (PS1,PM1,PM2,PP2,PP3,PP5)	YES	0	1
p.(Gly948Ala)	c.2843G>C	1	0	0.9999	Gly	3/280,952	NO		LP (PM1,PM2,PP2,PP3)	YES	1	0
p.(Gly939Ser)	c.2815G>A	1	0	0.9999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly927Glu)	c.2780G>A	1	0	1	Gly	None	NO		LP(PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly873Arg)	c.2617G>A	1	0	1	Gly	None	P, LP	Con-flicting*	P (PM1,PM2,PP2,PP3)	NO	1	1
GNp.(Gly864Arg)	c.2590G>A	1	0	1	Gly	1/31,324	LP, P	LP*	LP (PM1,PM2,PP2,PP3,PP5)	YES	0	2
p.(Gly861Glu)	c.2582G>A	1	0.001	0.9999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	1	0

p.(Gly840Arg)	c.2518G>A	1	0	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	1	0
p.(Gly837Arg)	c.2509G>C	1	0	1	Gly	None	Other x1, P		LP (PM1,PM2,PM5,PP2,PP3)	YES	0	2
p.(Gly825Ala)	c.2474G>C	0.125	0.048	0.9981	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	NO	0	1
GNp.(Gly816Val)	c.2447G>T	1	0	1	Gly	4/ 249,158	NO		LP (PM1,PM2,PP2,PP3)	YES	0	2
p.(Gly816Glu)	c.2447G>A	1	0	1	Gly	24/ 249,158	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
GNp.(Gly801Ala)	c.2402G>C	1	0	0.9999	Gly	1/ 249,424	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly801Glu)	c.2402G>A	1	0	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly798Ser)	c.2392G>A	1	0.005	0.9999	Gly	3/ 280,816	NO	VUS*	LP (PM1,PM2,PP2,PP3)	YES	0	3
p.(Gly783Arg)	c.2347G>A	1	0	1	Gly	None	NO	VUS*	LP (PM1,PM2,PP2,PP3)	YES	0	1
GNp.(Gly774Arg)	c.2320G>C	1	0	1	Gly	19/ 280,942	LP,P, VUS	LP**	LP (PM1,PM2,PP2,PP3,PP5)	YES	0	1
GNp.(Gly765Val)	c.2294G>T	1	0.009	1	Gly	9/ 249,576	NO		LP (PM1,PM2,PP2,PP3)	YES	0	2
p.(Gly751Arg)	c.2251G>A	1	0	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
GNp.(Gly748Ser)	c.2242G>A	0.954	0	1	Gly	9/ 280,758	NO	LP**	LP (PM1,PM2,PP2,PP3,PP5)	YES	2	3
GNp.(Gly734Ser)	c.2200G>A	1	0.009	0.9999	Gly	2/ 249,028	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly698Arg)	c.2092G>A	1	0	1	Gly	None	NO	VUS*	LP (PM1,PM2,PP2,PP3)	YES	1	0
p.(Gly660Asp)	c.1979G>A	1	0.006	0.9999	Gly	None	LP		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly651Cys)	c.1951G>T	1	0	0.9999	Gly	None	Other x1, LP		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly625Glu)	c.1874G>A	1	0.002	0.9999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	1	1
p.(Gly596Arg)	c.1786G>A	1	0	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly551TrpfsTer8)	c.1649dup	1			Gly	None	LP		LP (PM1,PM2,PP2,PP3)	YES	1	0

p.(Gly545Asp)	c.1634G>A	1	0	0.9999	Gly	None	B,VUS		LP (PM1,PM2,PP2,PP3)	NO	0	1
p.(Gly533Asp)	c.1598G>A	0.999	0	0.9982	Gly	None	LP, P	P*	LP (PM1,PM2,PP2,PP3,PP5)	YES	2	1
p.(Gly524Glu)	c.1571G>A	0.576	0.002	0.9999	Gly	1/ 249,290	LB		LP (PM1,PM2,PP2,PP3)	NO	0	1
p.(Gly503Trpfs Ter12)	c.1505dup	1			Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly500Val)	c.1499G>T	1	0.03	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly500Ala)	c.1499G>C	1	0.186 Tol)	0.9998	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	NO	0	1
p.(Gly479Arg)	c.1435G>C	0	0.466 (Tol)	0.9975 (Pol)	Gly	106/ 280,814		Con-flicting*	VUS (PM1,PM2,PP2, BP4)	NO	2	8
p.(Gly475Ala)	c.1424G>C	0.991	0	0.9999	Gly	None	NO	LP	LP (PM1,PM2,PM5,PP2,PP3)	YES	0	1
p.(Gly451Asp)	c.1352G>A	1	0.002	0.9999	Gly	None	Other x1 VUS		LP (PM1,PM2,PP2,PP3)	YES	1	0
p.(Gly442Ser)	c.1324G>A	0.997	0.06	0.9999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
GNp.(Gly426Arg)	c.1276G>A	1	0.001	0.9999	Gly	4/ 248,782	NO		LP (PM1,PM2,PP2,PP3)	YES	0	3
GNp.(Gly402Asp)	c.1205G>A	1	0.003	0.9999	Gly	5/ 247,742	NO		LP (PM1,PM2,PP2,PP3)	YES	0	3
p.(Gly382Ala)	c.1145G>C	0.999	0	0.9999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	1	0
p.(Gly379Arg)	c.1135G>A	1	0	0.9999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	1	0
p.(Gly379Ala)	c.1136G>C	0.999	0	0.9999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
GNp.(Gly373Glu)	c.1118G>A	1	0	0.9999	Gly	2/ 248,186	LP, VUS	P*	LP (PM1,PM2,PP2,PP3,PP5)	YES	2	2
p.(Gly335Val)	c.1004G>T	1	0	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	1	0
p.(Gly308Glu)	c.923G>A	1	0	0.9999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	2
p.(Gly291Glu)	c.872G>A	1	0.004	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly252Ser)	c.754G>A	1	0.009	0.9999	Gly	None	Other x 1 VUS		LP (PM1,PM2,PM5,PP2,PP3)	YES	0	1
GNp.(Gly190Arg)	c.568G>C	1	0	1	Gly	1/ 249,424	NO		LP (PM1,PM2,PP2,PP3)	YES	1	0
p.(Gly164Val)	c.491G>T	1	0	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1

p.(Gly161Val)	c.482G>T	1	0	1	Gly	4/ 249,086	VUS; Other x2 LP, VUS	LP*	LP (PM1,PM2,PM5,PP2,PP3,PP5)	YES	0	2
GNp.(Gly161Arg)	c.481G>C	1	0	1	Gly	3/ 280,524	NO	LP*	LP (PM1,PM2,PM5,PP2,PP3,PP5)	YES	0	2
p.(Gly143Asp)	c.428G>A	1	0	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
GNp.(Gly137Ser)	c.409G>A	1	0	1	Gly	1/ 248,804	LP, VUS		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly122Ser)	c.364G>A	1	0	0.9999	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
p.(Gly113Asp)	c.338G>A	1	0	0.9999	Gly	14/ 257,452	NO	VUS	LP (PM1,PM2,PP2,PP3)	YES	0	2
GNp.(Gly98Ser)	c.292G>A	1	0	0.9999	Gly	1/ 247,318	NO		LP (PM1,PM2,PP2,PP3)	YES	0	3
p.(Gly92Glufs Ter2)	c.275del	1	0	1	Gly	None	NO		LP (PM1,PM2,PP2,PP3)	YES	0	1
Total number with a predicted pathogenic variant with and without haematuria respectively											19	95
Total number with haematuria and without haematuria respectively											2,221	37,200

Variants with any evidence not consistent with pathogenicity are highlighted in blue. Otherwise notes are the same as for Suppl Table 3.

Suppl Table 6: Predicted pathogenic variants in *COL4A5* in the Exome Variant Server database

Protein consequence	Transcript	Affected individuals/ total allele count	PP2	SIFT	Mutation taster	Conserved	Previously reported; or substitution with other residue (LOVD)	ClinVar	Varsome	Alamut	Predicted pathogenic
[GN]p.(Gly42Ser)	c.124G>A	1M/ 10,563	Benign (0.013)	Damaging (0.047)	Disease causing (0.99)	Gly	NO		Likely pathogenic (PM1, PM2, PP2, PP3)	Weak	NO
[GN]p.(Gly624Asp)	c.1871G>A	1F/ 10,563	Prob damaging (0.999)	Damaging (0.119)	Disease causing (1.00)	Gly	Slajpah, 2007	**Pathogenic ****	Pathogenic (PM1, PP2, PP3, PP5)	Strong	YES
p.(Gly905Ser)	c.2713G>A	1F/10,563	Probably damaging 1.00	Damaging (0.00)	Disease causing (1.00)	Gly	NO		Likely pathogenic (PM1, PM2, PP2, PP3)	Strong	YES
[GN]p.(Gly953Val)	c.2858G>T	2M/ 10,563	Probably damaging 1.00	Damaging (0.002)	Disease causing (1.00)	Gly	Knebelmann, 1996	Conflicting B, LB, VUS	Benign (PM1, PP2, PP3,PP5, BS1, BS2)	Weak	NO
[GN]p.(Gly1074Arg)	c.3220G>C	1M/ 10,563	Benign (0.09)	Damaging (0)	Disease causing (0.999)	Gly	NO		Likely pathogenic (PM1, PM2,PP2,PP3)	Weak	NO
[GN]p.(Gly1244Ser)	c.3730G>A	1F/ 10,563	Benign (0.315)	Damaging (0.06)	Disease causing (1.00)	Gly	NO		Likely pathogenic (PM1, PM2, PM5, PP2,PP3)		NO
[GN]p.(Gly1424Ser)	c.4270G>A	1M/ 10,563	Probably damaging 1.00	Damaging (0)	Disease causing (1.00)	Gly	LOVD, p.(Gly1424Glu), Zhang, 2011		Likely pathogenic, PM2,PM3,PM5,PP2,PP3	Weak	YES

Evidence against pathogenicity is highlighted in blue. Three variants were predicted to be pathogenic in the cohort of 7042 individuals; p.(Gly624Asp), p.(Gly905Ser); and p.(Gly1424Ser). This cohort had no frameshift, termination codons or splice site variants. M male; F female. PP2 Polyphen-2, SIFT, Mutation taster computational tools assess pathogenicity. Conserved residue in vertebrates. ClinVar – star system used for quality of assertion * to ****. P Pathogenic, LP, Likely pathogenic, B – benign, LB likely benign, VUS – variant of uncertain significance. PVS, PM, PP, BS are ACMG/AMP criteria referring to criteria for P pathogenicity; and B benign nature; p.(Leu691Phefs*7)[1] where [1]read depth = 14 was excluded where all other read depths were > 30.

Suppl Table 7: Predicted pathogenic variants in *COL4A5* in the TOPMed database

Protein consequence	Transcript	Affected individuals/ total allele count = 125,568	PP2	SIFT	Mutation taster	Conserved	Previous reports of variant, or other variants at this location (LOVD)	ClinVar	Varsome	Predicted pathogenicity
In gnomAD	c.231+2T>C	1F			Disease-causing			Not found	Pathogenic (PVS1, PM2,PP3)	YES
	c.1024_1032+22delCCTGGACTTGTAAGTTTTTTTTTTTTAGTCT	1F			Disease-causing			Not found	Pathogenic (PVS1,PM2,PP3)	YES
p.(Gly57Arg)	c.169G>C	1F	1.00	Damaging (0)	Disease-causing	Gly	NO	Not found	Likely pathogenic (PM1,PM2,PP2,PP3)	YES
p.(Gly111Ser)	c.331G>A	1F	1.00	Damaging (0)	Disease-causing	Gly	NO	Not found	Likely pathogenic (PM1,PM2,PP2,PP3)	YES
^{GN}p.(Gly279Arg	c.835G>C	2F	0.999	Damaging (0.003)	Disease-causing	Gly	NO	Not found	Likely pathogenic (PM1,PM2,PP2,PP3)	YES
p.(Gly282Ser)	c.844G>A	1F	1.00	Damaging (0)	Disease-causing	Gly	NO	Not found	Likely pathogenic (PM1,PM2,PP2,PP3)	YES
^{GN}p.(Gly512Glu)	c.1535G>A	2F	1.00	Damaging	Disease-causing	Gly	NO	Not found	Likely pathogenic (PM1,PM2,PP2,PP3)	YES
^{GN}p.(Gly624Asp)	c.2073G>A	2M, 7F	0.99	Damaging (0)	Disease-causing	Gly	YES	Pathogenic **	Pathogenic (PM1, PP2, PP3, PP5)	YES
^{GN}p.(Gly752Val)	c.2255G>T	1F	1.00	Damaging (0.031)	Disease-causing	Gly	NO	Not found	Likely pathogenic (PM1,PM2,PP2,PP3)	YES
p.(Gly769Glu)	c.2306G>A	1F	1.00	Damaging (0)	Disease-causing	Gly	NO	Not found	Likely pathogenic (PM1,PM2,PM5,PP2,PP3)	YES
p.(Gly817Arg)	c.2449G>C	2M, 4F	1.00	Damaging (0.001)	Disease-causing	Gly	NO	Not found	Likely pathogenic (PM1,PM2,PP2,PP3)	YES
p.(Gly905Ser)	c.2713G>A	2F	1.00	Damaging (0.001)	Disease-causing	Gly	NO	Not found	Likely pathogenic (PM1,PM2,PM5,PP2,PP3)	YES

p.(Gly973Ser)	c.2917G>A	1F	1.00	Damaging (0)	Disease-causing	Gly	NO	Not found	Pathogenic (PVS1, PM1,PM2,PM5, PP2,PP3)	YES
p.(Gly1069Val)	c.3206G>T	1F	1.00	Damaging (0)	Disease-causing	Gly	VUS	Conflicting *	Likely pathogenic (PM1,PM2,PP2,PP3)	NO
p.(Gly1113Ala)	c.3338G>C	1F	1.00	Damaging (0.01)	Disease-causing	Gly	NO	Not found	Likely pathogenic (PM1,PM2,PP2,PP3)	YES
p.(Gly1119Ala)	c.3356G>C	1F	0.999	Damaging (0)	Disease-causing	Gly	NO	Not found	Likely pathogenic (PM1,PM2,PP2,PP3)	YES
p.(Gly1153Arg)	c.3457G>C	3M, 2F	0.077	Tol (0.515)	Pol (0.9981)	Gly	NO	Not found	VUS (PM1,PP2,BP4)	NO
p.(Gly1303Cys)	c.3907G>T	1M, 1F	1.00	0	Disease-causing	Gly	NO	Not found	Likely pathogenic (PM1,PM2,PP2,PP3)	YES
p.(Gly1330Ser)	c.3988G>A	1F	0.998	0	Disease-causing	Gly	NO	Not found	Likely pathogenic (PM1,PM2,PP2,PP3)	YES
p.(Gly1339Ala)	c.4016>C	1M, 1F	1.00	Damaging (0.007)	Disease-causing	Gly	NO	Not found	Pathogenic (PM1,PM2,PM5,PP2 ,PP3)	YES
[GN]p.(Gly1424Ser)	c.4270G>A	3F	Probably damaging 0.998	Damaging (0)	Disease causing (1.00)	Gly	LOVD, p.(Gly1424Glu), Zhang, 2011	Not found	Likely pathogenic (PM1,PM2,PM5,PP2 ,PP3)	YES
		6M,34F in 83,712 = 1/2093								

GN also in gnomAD

Suppl Table 8: Mitigating features for the clinical effects of predicted pathogenic *COL4A5* variants resulting in position 1 Gly substitutions in gnomAD: associations with gender, location, and replacement residues

Mutation	Alleles	Gender	Exons 1-20	Adjacent to non-collagenous domain or interruption	Substitution with Ala, Ser, Cys
p.(Gly69Arg)	1	F	Yes		
p.(Gly84Glu)	1	F	Yes		
p.(Gly105Ala)	1	M	Yes		Yes
p.(Gly114Ala)	1	F	Yes		Yes
p.(Gly126Glu)	1	F	Yes		
p.(Gly201Ala)	1	M	Yes		Yes
p.(Gly279Ala)	1	M	Yes		Yes
p.(Gly283Val)	1	F	Yes	Yes	
p.(Gly286Cys)	1	F	Yes		Yes
p.(Gly356Glu)	1	M	Yes	Yes	
p.(Gly356Ala)	1	F	Yes	Yes	Yes
p.(Gly512Glu)*	**1**	**M**			
p.(Gly536Ser)	1	F			Yes
p.(Gly624Asp)	16	M (n=4), F (n=12)		Yes	
p.(Gly656Ser)	1	M		Yes	Yes
p.(Gly702Ser)	1	F			Yes
p.(Gly752Val)	2	M (n=2)		Yes	
p.(Gly787Ala)	1	F			Yes
p.(Gly822Glu)*	**1**	**M**			
p.(Gly893Val)	1	F			
p.(Gly967Arg)	1	F			
p.(Gly1054Asp)*	**1**	**M**			
p.(Gly1134Cys)	1	F			Yes
p.(Gly1170Ser)	1	F			Yes
p.(Gly1185Ser)	1	F			Yes
p.(Gly1249Arg)	1	F		Yes	
p.(Gly1282Glu)	**1**	**M**			
p.(Gly1300Ala)	1	F			Yes
p.(Gly1303Ala)	1	M			Yes
p.(Gly1321Val)	1	F			
p.(Gly1324Glu)	1	F			
p.(Gly1333Cys)	1	M			Yes
p.(Gly1394Cys)	2	F (n=2)			Yes
p.(Gly1424Ser)	1	F			Yes
N=34	51	17M, 34F	N=11	N=7	N=18

M male, F female * variants where there were no biochemical features potentially mitigating clinical phenotype

Suppl Table 9: Variants in *COL4A3* and *COL4A4* in gnomAD with inconsistent assessments subsequently excluded from population frequency studies

hg19	rs ID	Variant	Trans cript	PP2	SIFT	MT	Cons erved in verte-brates	Clin Var	Varsome	gnomad	Cohort size	A	L	AJ	EA	F	EUR	O	SA
COL4A3																			
228102723	rs13424243	p.(Gly43Arg)	c.127G>A	1	DEL	P	Ser	Benign	VUS (PM1,PM2,PP2,BP4)	3	280794	0	0	1	0	0	2	0	0
228102723	rs13424243	p.(Gly43Arg)	c.127G>C	1	DEL	P	Ser	Benign	Benign (PM1,PP2,BA1,BP4,BP6)	80620	280794	7524	7749	3493	740	8390	46200	2242	4282
228102723	rs13424243	p.(Gly43Trp)	c.127G>T	1	DEL	P	Ser	Benign	VUS (PM1,PM2,PP2,BP4)	80623	280794	1	0	0	0	0	1	0	0
228102724	rs776294835	p.(Gly43Glu)	c.128G>A	1	DEL	P	Ser	Not found	VUS (PM1,PM2,PP2,BP4)	3	249528	3	0	0	0	0	0	0	0
228102732	rs200866082	p.(Gly46Arg)	c.136G>A	1	DEL	DC	Gly	Likely benign	Likely pathogenic (PM1,PM2,PP2,PP3,BP6)	61	280882	57	3	0	0	0	0	1	0
228131759	rs745472969	p.(Gly487Arg)	c.1459G>C	1	DEL	0.606 - P	Gly	VUS	Likely pathogenic (PM1,PM2,PP2,PP3)	1	249514	0	0	0	0	0	1	0	0
228142243	rs1331805495	p.(Gly700Val)	c.2099G>T	0.977	TOL (0.06)	DC	Gly	Not found	Likely pathogenic (PM1,PM2,PP2,PP3)	1	245134	0	0	0	0	0	1	0	0
228145668	rs774838919	p.(Gly812Ser)	c.2434G>A	0.339	TOL (0.21)	P	Cys	Not found	VUS (PM1, PM2,PP2,BP4)	10	178112	0	4	0	0	0	5	1	0
228142227	rs200287952	p.(Gly695Arg)	c.2083G>A	1	TOL 0.08	DC	Gly	Pathogenic	Pathogenic (PM1,PM2,PP2,PP3,PP5)	31	278152	1	0	0	0	0	27	3	0
228104886	rs184730597	p.(Gly58Ser)	c.172G>A	1	DEL	DC	Gly	VUS	Likely pathogenic (PM1,PM2,PP2,PP3)	6	243384	0	3	0	0	0	2	1	0
228112275	rs775373641	p.(Gly148Val)	c.443G>T	1	DEL	DC	Gly	VUS	Likely pathogenic (PM1,PM2,PP2,PP3)	4	280874	0	0	1	0	0	3	0	0
228113159	rs764451365	p.(Gly157Arg)	c.469G>C	1	DEL	DC	Gly	VUS	Likely pathogenic (PM1,PM2,PP2,PP3)	4	249350	0	0	0	0	0	1	1	2
228131759	rs745472969	p.(Gly487Ser)	c.1459G>A	1	DEL	DC	Gly	VUS	Likely pathogenic (PM1,PM2,PM5,PP2,PP3)	31	280912	0	15	0	0	0	10	1	5
228149007	rs1265432530	p.(Gly943Arg)	c.2827G>A	1	DEL	DC	Gly	VUS	Likely pathogenic (PM1,PM2,PP2,PP3)	2	249332	0	0	0	1	0	1	0	0
228168602	rs372237167	p.(Gly1328Ala)	c.3983G>C	1	DEL	DC	Gly	VUS	Likely pathogenic (PM1,PM2,PP2,PP3)	1	249382	0	0	0	0	0	0	1	0
COL4A4																			
227984654	rs1370340334	p.(Gly110Ala)	c.329G>C	1	TOL	DC	Gly	Not found	Likely pathogenic (PM1,PM2, PP2,PP3)	1	221310	0	0	0	0	0	1	0	0
227984645	rs766085522	p.(Gly113Asp)	c.338G>A	1	TOL	DC	Gly	Not found	Likely pathogenic (PM1,PM2, PP2,PP3)	14	257452	9	5	0	0	0	0	0	0
227983440	rs377511303	p.(Gly137Asp)	c.410G>A	1	DEL	DC	Gly	VUS	Likely pathogenic (PM1,PM2, PP2,PP3)	2	248802	2	0	0	0	0	0	0	0
227983392	rs773360119	p.(Gly153Val)	c.458G>T	0.712	DEL	DC	Gly	Not found	VUS (PM1,PM2,PP2,BP4)	2	249180	0	0	0	0	0	2	0	0

227958987	rs1026613471	p.(Gly408Glu)	c.1223G>A	1	DEL	DC	Gly	Conflicting; Benign	Likely pathogenic (PM1,PM2,PP2,PP3)	6	248574	0	0	5	0	0	1	0	0
227954608	rs202210475	p.(Gly479Arg)	c.1435G>C	0	**TOL**	**P**	Gly	Conflcting;VUS	VUS (PM1,PM2,PP2,BP4)	106	280814	0	6	75	0	0	16	8	1
227946893	rs1800516	p.(Gly545Ala)	c.1634G>C	1	DEL	**P**	Gly	Benign	Benign (PM1,PP2, PP3,BS1,BS2,BP6)	7694	280884	798	675	446	0	548	4724	262	241
227924914	rs781014928	p.(Gly701Val)	c.2102G>T	0.82	**TOL**	DC	Gly	Not found	Likely pathogenic (PM1,PM2, PP2,PP3)	1	248786	0	0	0	0	0	0	0	1
227922308	rs760803228	p.(Gly798Ser)	c.2392G>A	1	DEL	DC	Gly	VUS	Likely pathogenic (PM1,PM2,PP2,PP3)	3	280816	0	0	0	0	0	3	0	0
227920820	rs752296059	p.(Gly853Arg)	c.2557G>A	0	**TOL**	**P**	Gly	Not found	VUS (PM1,PM2,PP2,BP4)	1	249324	0	0	0	1	0	0	0	0
227915847	rs13027659	p.(Gly999Glu)	c.2996G>A	1	DEL	DC	Gly	Conflicting; benign; likely benign	VUS (PM1, PP2,PP3,PP5, BS1,BS2)	3321	280922	68	278	27	0	228	2508	104	108
227915821	rs371172166	p.(Gly1008Arg)	c.3022G>A	1	DEL	DC	Gly	VUS	Likely pathogenic (PM1,PM2,PP2,PP3)	9	280942	6	2	0	0	0	1	0	0
227915799	rs764323652	p.(Gly1015Glu)	c.3044G>A	1	DEL	DC	Gly	Conflicting; Likely pathogenic; VUS	Likely pathogenic (PM1,PM2,PP2,PP3)	14	280944	0	0	0	0	0	14	0	0
227915754	rs772699709	p.(Gly1030Val)	c.3089G>T	1	DEL	DC	Gly	**VUS**	Likely pathogenic (PM1, PM2,PM5,PP2,PP3,PP5)	1	249562	0	0	0	0	0	1	0	0
227983440	rs377511303	p.(Gly137Asp)	c.410G>A	1	DEL	DC	Gly	**VUS**	Likely pathogenic (PM1,PM2, PP2,PP3)	2	248802	2	0	0	0	0	0	0	0
227876903	rs779769090	p.(Gly1443Arg)	c.4327G>A	**0.005**	**DEL (0.22)**	**P**	Gly	Not found	VUS (PM1,PM2,PP2,BP4)	1	249498	0	0	0	0	0	1	0	0
227876903	rs779769090	p.(Gly1443Arg)	c.4327G>C	**0.005**	**DEL (0.22)**	**P**	Gly	Not found	VUS (PM1,PM2,PP2,BP4)	13	249498	0	0	0	0	0	0	0	13
227875175	rs1287040507	p.(Gly1459Val)	c.4376G>T	**0.627**	**DEL**	**P**	Gly	Not found	VUS (PM1,PM2,PP2,BP4)	2	274180	0	0	0	0	0	2	0	0

Evidence supporting pathogenicity is highlighted in red; and not supporting pathogenicity is in blue. PP2, Polyphen-2, SIFT, MT Mutation taster. PP2 scores >0.8 indicate Pathgoenic or Likely Pathogenic. SIFT scores DEL for deleterious, TOL for tolerated. MT scores DC for disease-causing and P for polymorphism. Varsome online tool to assess ACMG criteria (varsome.com), gnomad variant database used to assess variant prevalence (approximate population n= 256, 562 for *COL4A3*, and 233,529 for *COL4A4*). Cohort size varied for different variants, A African (n = 15,944) L Latino (n=32,544), AJ Ashenazi Jewish (n=9528), EA East Asian (n=17,021), F Finnish (n=21,559), Eur European (non-Finnish, n=111,786), Other (n=6112), SE South Asian (n=28,471)

Of especial interest were p.(Gly545Ala) and p.(Gly999Glu) in *COL4A4*, which were not predicted pathogenic and excluded from further analysis. The p.(Gly545Ala) variant was found 7,626 times in gnomAD, including 145 times in the homozygous form. It was considered pathogenic in two out of three computational tools, affected a conserved residue, but had Benign assessments from ClinVar and Varsome (based on one moderate (PM1) and 2 supporting criteria for pathogenic (PP2, PP3) and two strong (BS1,BS2) and one supporting criteria for benign (BP6)).

The p.(Gly999Glu) variant was found 3,259 times, including 33 times in the homozygous form. It was considered pathogenic in all three computational tools, and affected a conserved residue, but was considered a Variant of Uncertain Significance by both ClinVar and Varsome based on one moderate (PM1), and three supporting (PP2,PP3 and PP5) criteria for pathogenicity, and two strong criteria for benign (BS1 and BS2).

Suppl Table 10: Predicted pathogenic founder variants in *COL4A3* and *COL4A4* in different ancestries in gnomAD

Gene	Variant	Transcript	Total	African	Latino	Ashkenazi	East Asian	Eur (Finnish)	Eur (non-Finnish)	Other	South Asian
COL4A3	None										
COL4A4	p.(Gly445Ala)	c.1334G>C	**22**	**19**					**3**		
	p.(Gly481Ser)	c.1441G>A	**21**		**21**						
	p.(Gly816Glu)	c.2447G>A	**24**				**24**				
	p.(Gly1005Glu)	c.3014G>A	**25**	**1**	**23**				**1**		
	p.(Gly1178Ser)	c.3532G>A	**24**		**3**			**3**	**16**	**2**	
	p.(Gly774Arg)	c.2320G>C	**19**					**1**	**5**	**13**	
	p.(Ser969Ter)	c.2906C>G	**18**	**1**					**16**	**1**	

Variants were arbitrarily designated founder variants if they resulted in a position 1 Gly substitution, frameshift, nonsense or canonical splice variant that was predicted to be pathogenic and occurred ≥ 18 times in gnomAD

Suppl Table 11: Estimated population frequencies for predicted pathogenic heterozygous, compound heterozygous, and digenic variants

COL4A3	*COL4A4*	Classification	Frequency	Total population frequency*
+/+	+/+	Wildtype	9.91×10^{-1}	9.91×10^{-1} or 99 in 100
+/+	+/-	Heterozygous predicted pathogenic	2.45×10^{-3}	9.41×10^{-3} or one in 106
+/+	-/+	Heterozygous predicted pathogenic	2.45×10^{-3}	
+/-	+/+	Heterozygous predicted pathogenic	2.26×10^{-3}	
-/+	+/+	Heterozygous predicted pathogenic	2.26×10^{-3}	
+/-	+/-	Digenic predicted pathogenic variants	5.58×10^{-6}	2.23×10^{-5} or one in 44,793
+/-	-/+	Digenic predicted pathogenic variants	5.58×10^{-6}	
-/+	+/-	Digenic predicted pathogenic variants	5.58×10^{-6}	
-/+	-/+	Digenic predicted pathogenic variants	5.58×10^{-6}	
+/+	-/-	Compound heterozygous predicted pathogenic variants	6.06×10^{-6}	1.13×10^{-5} or one in 88,866 individuals
+/-	-/-	Compound heterozygous predicted pathogenic variants	1.38×10^{-8}	
-/+	-/-	Compound heterozygous predicted pathogenic variants	1.38×10^{-8}	
-/-	+/+	Compound heterozygous predicted pathogenic variants	5.14×10^{-6}	
-/-	+/-	Compound heterozygous predicted pathogenic variants	1.27×10^{-8}	
-/-	-/+	Compound heterozygous predicted pathogenic variants	1.27×10^{-8}	
-/-	-/-	Compound heterozygous predicted pathogenic variants	3.14×10^{-11}	

+ wildtype, - variant allele. The frequencies \have been calculated from the sum of the likelihood of the individual combinations of alleles. These calculations are based on the number of predicted pathogenic

variants in the mean alleles examined that is 559/245,889 for *COL4A3* and 577/233,916 for *COL4A4* and assume that *COL4A3* and *COL4A4* variants occur together independently. If all predicted pathogenic variants are pathogenic, then heterozygous predicted pathogenic variants correspond to Thin basement membrane nephropathy or AD Alport syndrome; digenic predicted pathogenic variants correspond to digenic Alport syndrome; and compound heterozygous pathogenic variants correspond to the compound heterozygous form of AR Alport syndrome. It is not possible to use this method to calculate how commonly homozygous forms of predicted pathogenic variants occur because they are usually the result of consanguineous relationships and do not occur by chance.

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