Supplemental Material Table of Contents

CONSORTIUM NAME

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Supplemental Table 1. NC1 domain locations within the six human collagen IV α-chains.

Chain	Isoform	NC1 domain (residues)	NCBI transcript reference sequence	NCBI protein reference sequence
α1	1	1439 – 1669	NM_001845.6	NP_001836.3
α2	1	1484 – 1712	NM_001846.4	NP_001837.2
α3	1	1437 – 1670	NM_000091.5	NP_000082.2
α4	1	1458 – 1690	NM_000092.5	NP_000083.3
α5	2	1461 – 1691	NM_033380.3	NP_203699.1
α6	A	1462 – 1691	NM_001847.4	NP_001838.2

Supplemental Table 2. Splicing predictions for all exonic single-nucleotide substitutions located within 3 bases of a canonical splice site.

Gene	Nucleotide change	Expected protein change	Type	MES score (Mutant)	MES score (WT)	MES score change (%)	Predicted to affect splicing	Database
COL4A5	c.4528G>C	p.Gly1510Arg	Splice donor site	2.24	6.04	-62.91	Yes	ClinVar
COL4A5	c.4708T>C	p.Cys1570Arg	Splice acceptor site	9.72	10.91	-10.91	No	LOVD
COL4A5	c.4709G>T	p.Cys1570Phe	Splice acceptor site	10.19	10.91	-6.60	No	ClinVar
COL4A5	c.4709G>C	p.Cys1570Ser	Splice acceptor site	10.93	10.91	0.18	No	LOVD, ClinVar
COL4A5	c.4709G>A	p.Cys1570Tyr	Splice acceptor site	10.45	10.91	-4.22	No	LOVD
COL4A5	c.4819A>G	p.Met1607Val	Splice donor site	3.10	3.85	-19.48	Yes	LOVD
COL4A5	c.4823A>G	p.His1608Arg	Splice acceptor site	11.66	11.30	3.19	No	gnomAD
COL4A5	c.4993A>T	p.Ser1665Cys	Splice donor site	5.75	9.65	-40.41	Yes	LOVD

Grey rows indicate variants predicted to affect normal splicing. Known exonic splicing variants are not shown. MES, MaxEntScan; WT, Wild type.

Supplemental Table 3. Categorisation of all possible unique collagen IV α5 NC1 missense variants.

a) All variants

		Residue:		
		Conserved[a]	Not conserved	Total
Variant:	Any structural damage	196	78	274
	No structural damage	404	638	1042
	Total	600	716	1316

p<0.001, OR (95% CI)=3.96 (2.94, 5.38)

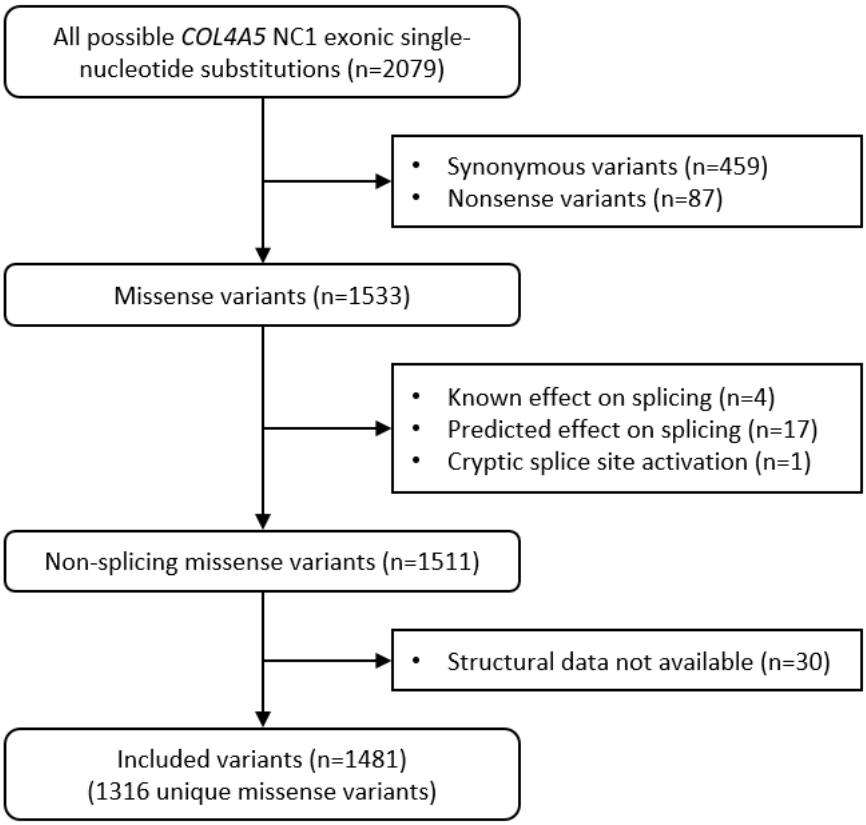
b) Excluding Cys variants

		Residue:		
		Conserved[a]	Not conserved	Total
Variant:	Any structural damage	124	78	202
	No structural damage	404	638	1042
	Total	528	716	1244

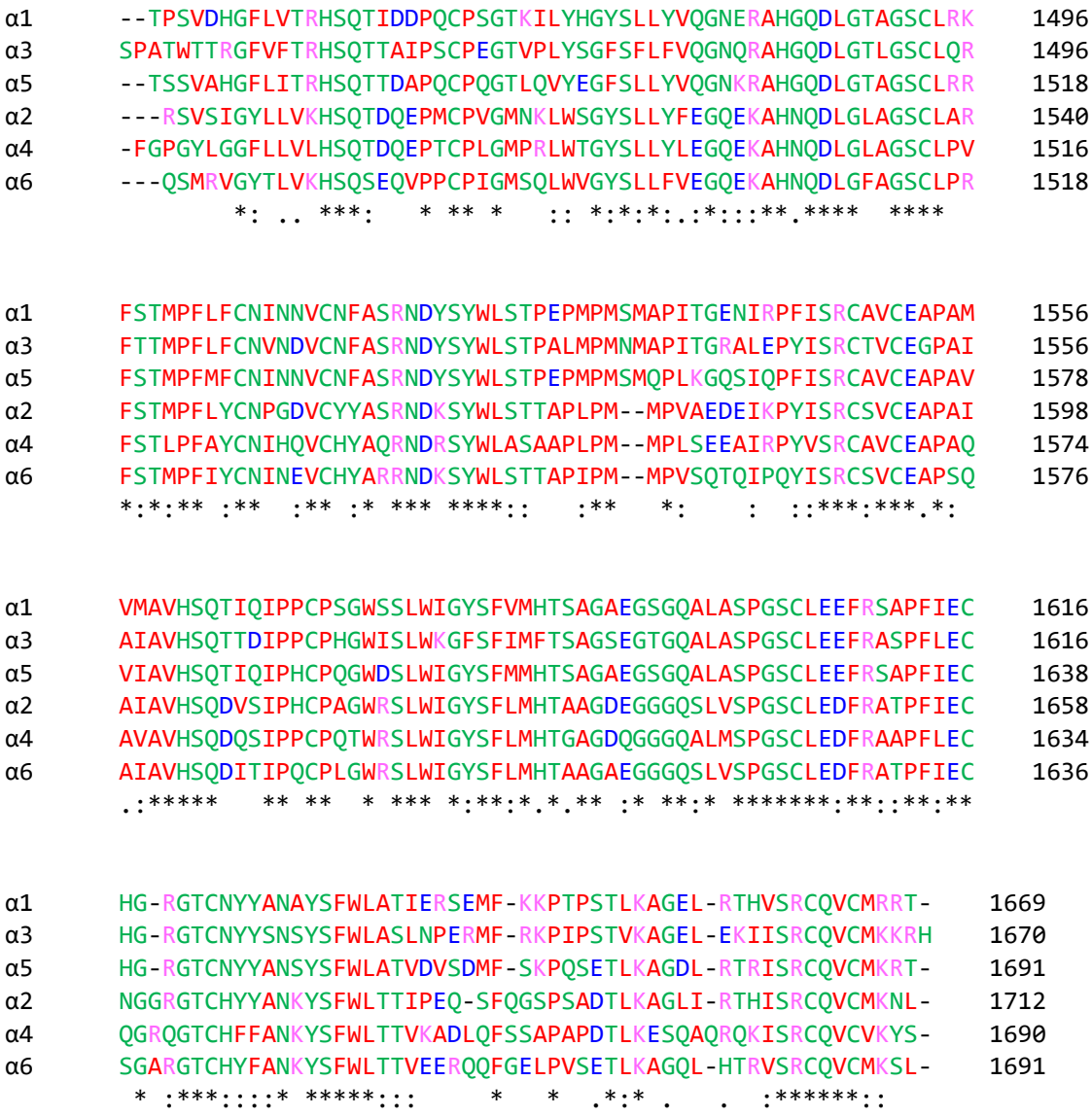
p<0.001, OR (95% CI)=2.51 (1.82, 3.47)

OR (95% CI), Odds ratio (95% confidence interval).

[a] Residues fully conserved in all six human collagen IV α-chains.



Supplemental Figure 1. Variant inclusion flowchart for the theoretical cohort comprising all possible collagen IV α5 NC1 missense variants. This was used to calculate the expected frequencies of structural changes for variants reported in LOVD.



Supplemental Figure 2. Multiple sequence alignment of the six human collagen IV α-chains. Chains are grouped into α1-like chains (α1, α3, α5; above) and α2-like chains (α2, α4, α6; below). Positions with a single, fully conserved residue are indicated with an asterisk (*). Alignment was performed using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/).