

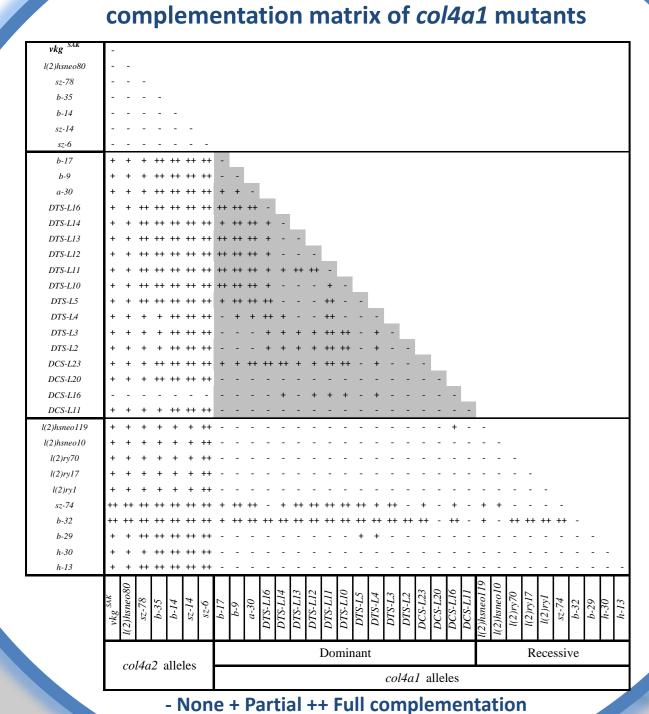
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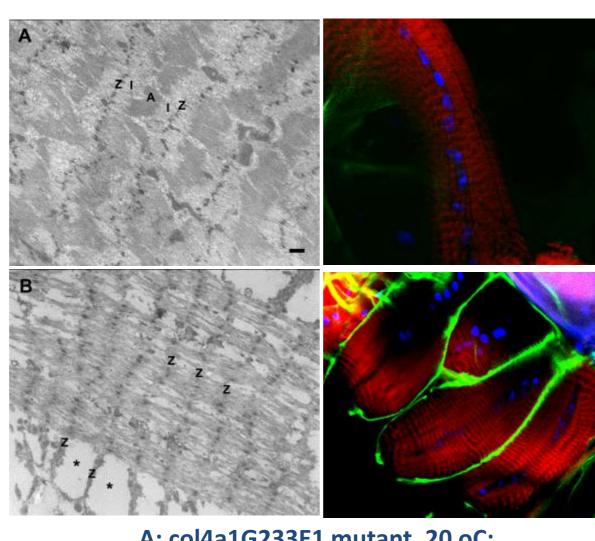
An Animal Model for Novel Human Diseases Associated with Mutations of Type IV collagen COL4A1 and COL4A2

Inter- and intragenic



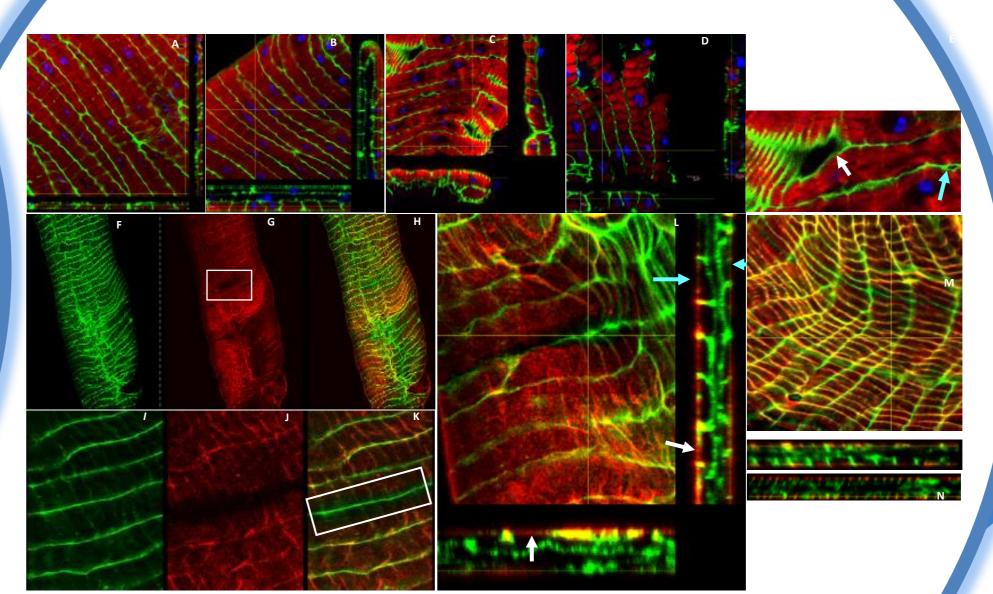
Electron microscopic image

of the larval body wall muscle and desorganisation of the indirect flight muscle in col4a1G552D2 mutant



A: col4a1G233E1 mutant, 20 oC; B: col4a1G233E1 mutant, 29 oC **Control / Mutant**

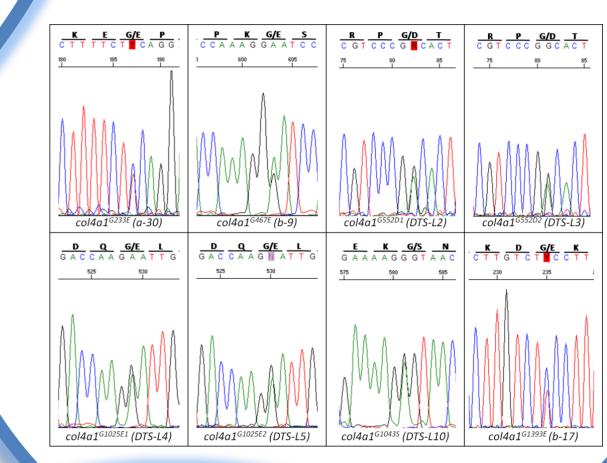
Myofibrillar pathology of the col4a1G552D2 mutant in striated oviductal muscle



Green: Basement membrane, stained by the COL4A2::GFP fusion protein

Red: in A-E: F-actin staining by phalloidin-Texas red conjugate Red in F-O: Distribution of laminin gamma-1 in the oviducts Blue: Nuclei stained by DAPI

Glycine substitutions in col4a1 variants



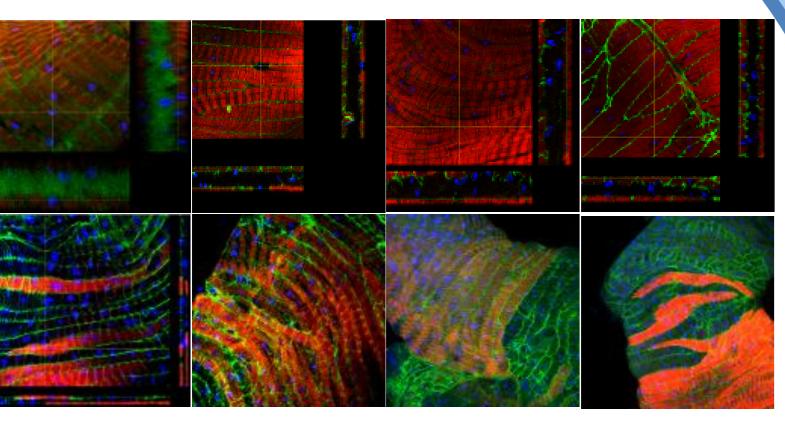
DNA-array-expressional analysis in the *col4a1*^{G552D2} mutant: Upregulation of immune functions suggest chronic inflammation at restrictive temperature

FC Ore	FC col4a1 ^{G552D2}	Gene Symbol	Gene Name
0,107246	36,62861	Mtk	Metchnikovin
0,166082	14,3166	CecA2	Cecropin A2
0,666819	2,394327	deltaTry	Delta trypsin
0,075071	16,3632	DptB	Diptericin B
0,177677	9,799489	AttA	Attacin A
0,984467	2,884275	LysB	Lysozyme B
0,216459	25,41022	CecC	Cecropin C
0,080118	10,76713	AttC	Attacin C
			(serine-type
0,8118	4,385851	CG18563	endopeptidase)
0,766593	3,521254	CG13905	(Malpighi specific)
1,265216	0,308932	CG12807	Serpin 85F

Blue: downregulated Red: upregulated

Green: defense response

Onset of muscular dystrophy with low progressivity in col4a1 mutants



COL4A2::GFP F-actin Nuclei

Muscle fibres do not round up and keep their angular ends Fibres do not detach laterally

Basement membrane scaffold remains intact Fibrotic areas are not filled by COL4A2::GFP Muscle fibre degeneration occurres in large areas

Summary

The systemic phenotype Hereditary Angiopathy, Nephropathy, Aneurysms, and muscle Cramps (HANAC) is associated with glycine substitutions localized within the CB3[IV] fragment of COL4A1, the major integrin-binding-site

Different genetic variants of *COL4A1* cause myopathy in mice and Walker-Warburg Syndrome in humans

However, in depth studies of the mutant muscle tissue are lacking and significant details of the underlying pathomechanism remain unknown

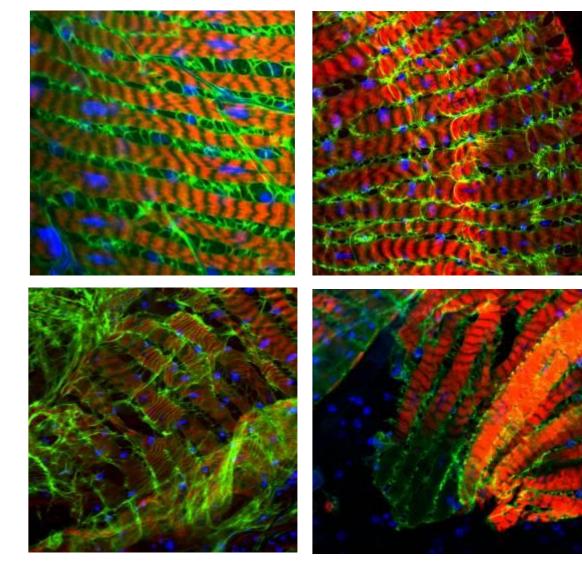
Our results demonstrate that the conditional, temperaturesensitive *Drosophila col4a1* mutants show intra- and extracellular disorganisation of fibres in multiple muscle types

Myopathic changes fulfill the criteria of the onset of muscular dystrophy. Slowly progressive form is observed in mutants affecting mainly the COL4A1 C-terminal, whereas rapidly progressive muscular dystrophy onsets in COL4A1 variants affecting integrin-binding-sites and trigger furin-mediated myofibrillar proteolysis

Further elements of the systemic phenotype include the gut and the Malpighian tubules that correspond to a single glomerolus

Transcriptome analysis of the col4a1 mutant flies revealed upregulation of the immune system and chronic inflammation

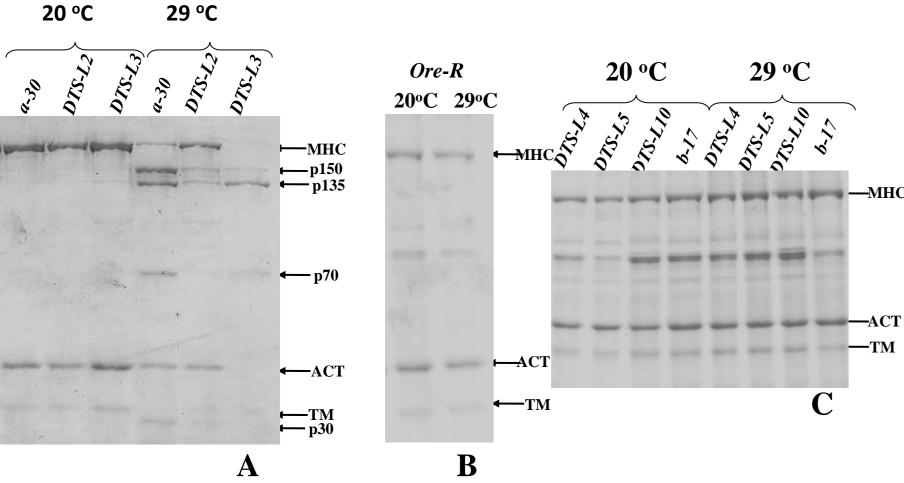
Smooth muscle cells of the gut: Cellular and extracellular degradation in col4a1G552D2 mutant



wt control; *col4a1*^{G552D2} 20 °C; col4a1^{G552D2} 29 °C; col4a1^{G552D2} 29 °C Red: F-actin

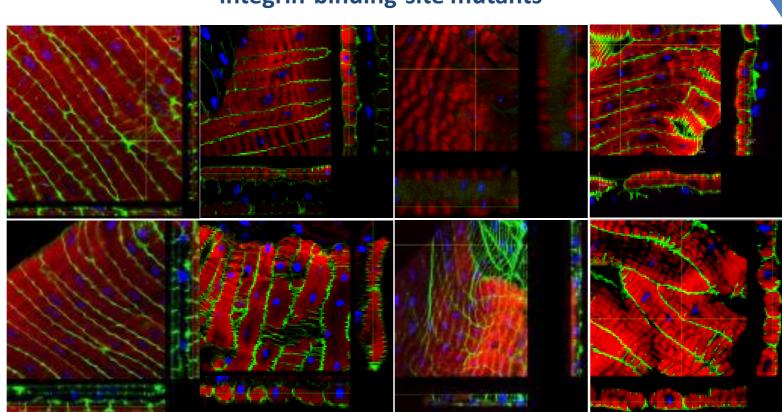
Onsets in ~28 days at 29 °C

Myofibrillar proteolysis in integrin-binding col4a1 mutants



Affected alleles: col4a1G233E; col4a1G552D1; col4a1G233E (Integrin-binding) Unaffected alleles: col4a1^{G1025E1}; col4a1^{G1025E2}; col4a1^{G1043S}; col4a1^{G1393E}

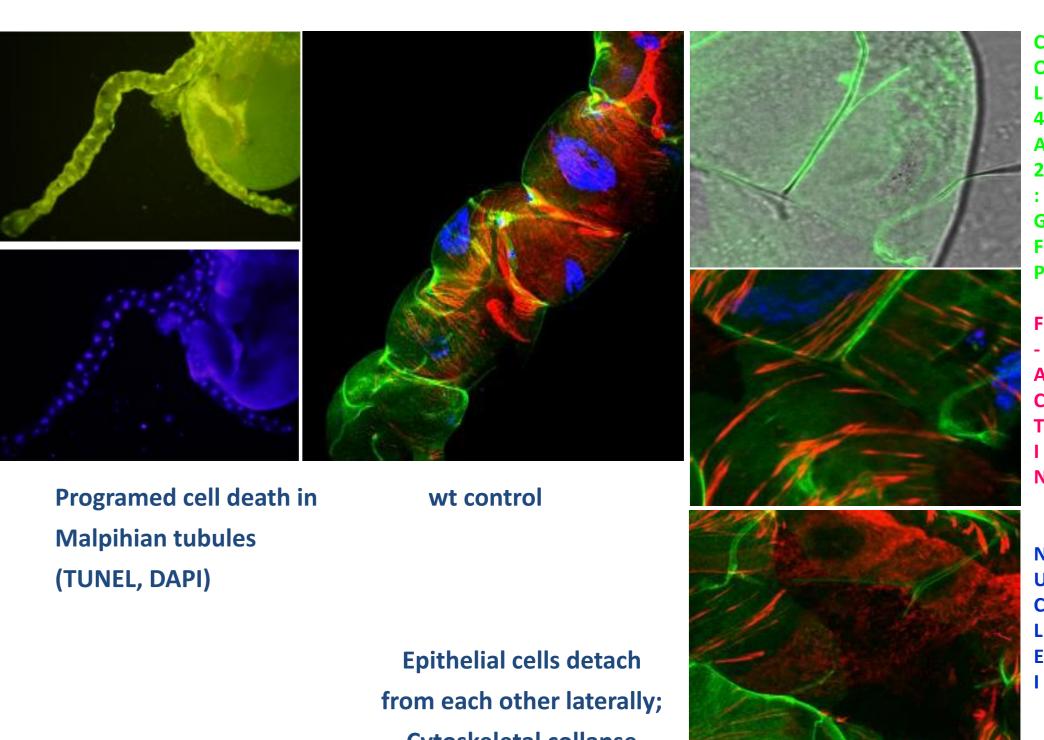
Rapid onset of muscular dystrophy in integrin-binding-site mutants



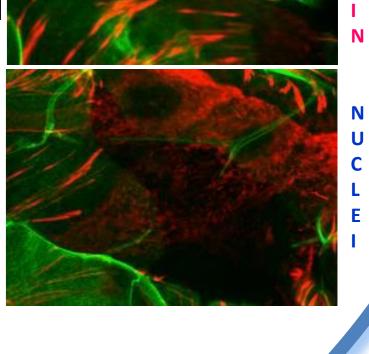
COL4A2::GFP F-actin Nuclei

Muscle fibres round up at their angular ends Fibres detach laterally Fibrotic areas are filled by COL4A2::GFP Muscle fibres degenerate in large areas Onsets in ~14 days at 29 °C

Malpighi-pathology in the col4a1G552D2 mutant



Cytoskeletal collapse



National Development Agency www.ujszechenyiterv.gov.hu 06 40 638 638

