Mutations in ANO5 represent a common cause of non-dysferlin LGMD2B and Miyoshi myopathy

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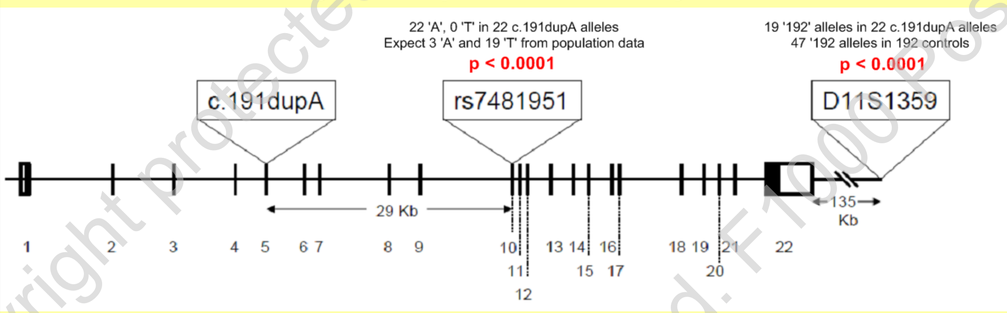
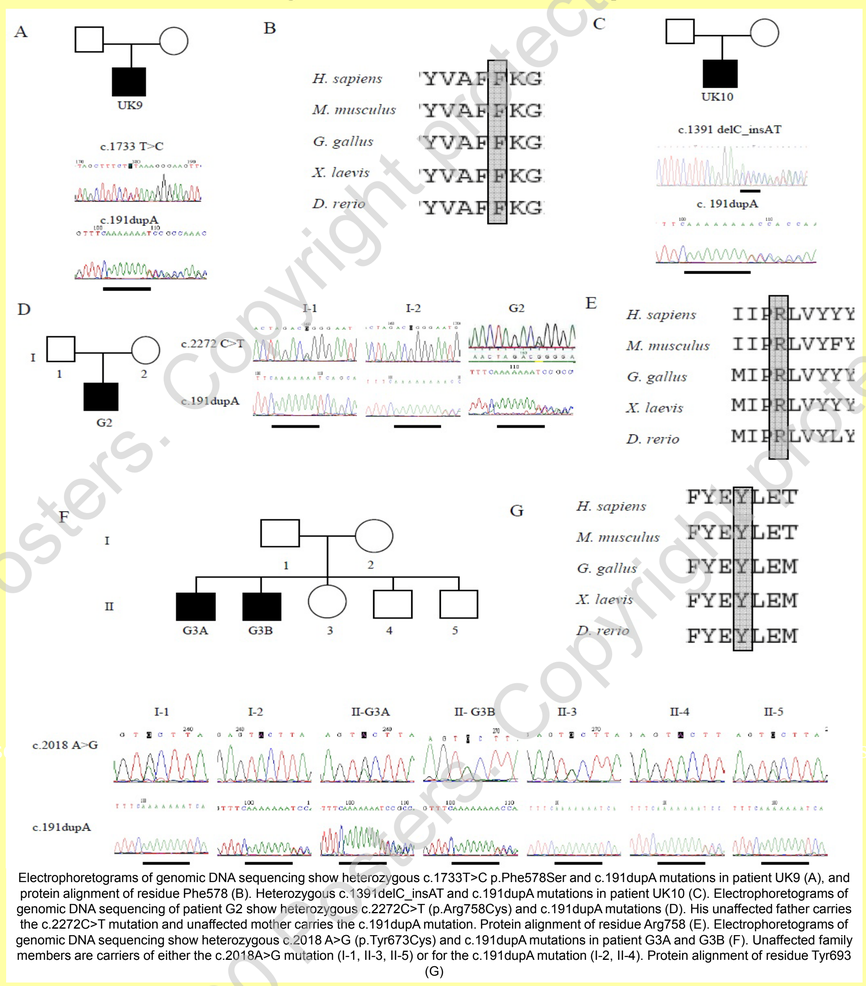
Introduction

- LGMD2B and Miyoshi Myopathy are overlapping disorders caused by mutations in Dysferlin
- However, there is also genetic heterogeneity and recently mutations in ANO5 have been identified in a several families with a phenotype resembling LGMD2B
- There is a common mutation in ANO5 segregating in the Northern European population and we have now shown that this mutation is in linkage disequilibrium with adjacent markers, indicating a founder effect.
- The phenotype of this prevalent cause of LGMD is described below.

Linkage disequilibrium analysis



Molecular analysis of non-c.191dupA mutations



Electrophoretograms of genomic DNA sequencing show heterozygous c.1733T>C p.Phe578Ser and c.191dupA mutations in patient UK9 (A), and protein alignment of residue Phe578 (B). Heterozygous c.1391delC_insAT and c.191dupA mutations in patient UK10 (C). Electrophoretograms of genomic DNA sequencing of patient G2 show heterozygous c.2272C>T (p.Arg758Cys) and c.191dupA mutations (D). His unaffected father carries the c.2272C>T mutation and unaffected mother carries the c.191dupA mutation. Protein alignment of residue Arg758 (E). Electrophoretograms of genomic DNA sequencing show heterozygous c.2018 A>G (p.Tyr673Cys) and c.191dupA mutations in patient G3A and G3B (F). Unaffected family members are carriers of either the c.2018A>G mutation (I-1, II-3, II-5) or for the c.191dupA mutation (I-2, II-4). Protein alignment of residue Tyr693 (G)

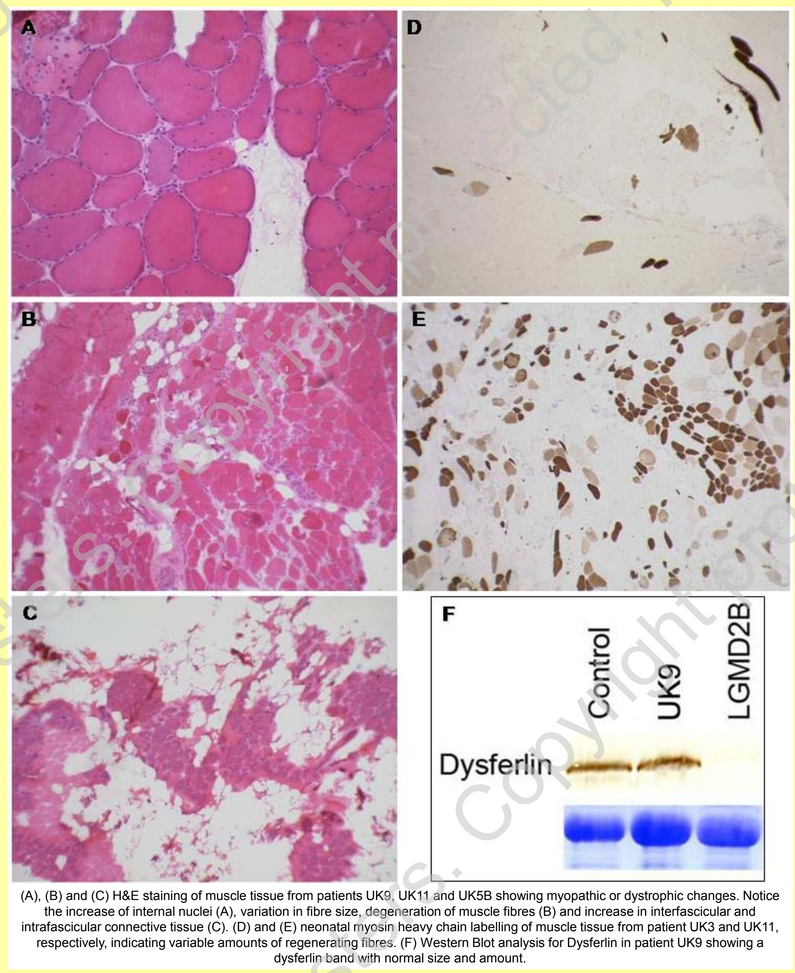
Results

- In 20 patients from 15 families, we identified the c.191dupA mutation, giving a detection rate in our phenotypically suggestive cohort of approximately 32%
- 15 of the 20 patients were homozygous for c.191dupA and analysis of the pathogenicity of the other ANO5 mutation in the remaining patients is given in the figure below.
- There is a striking gender predominance with only 2/20 ANO5 patients being female.

For MRI data see poster by Sarkozy *et al*, P4.27.

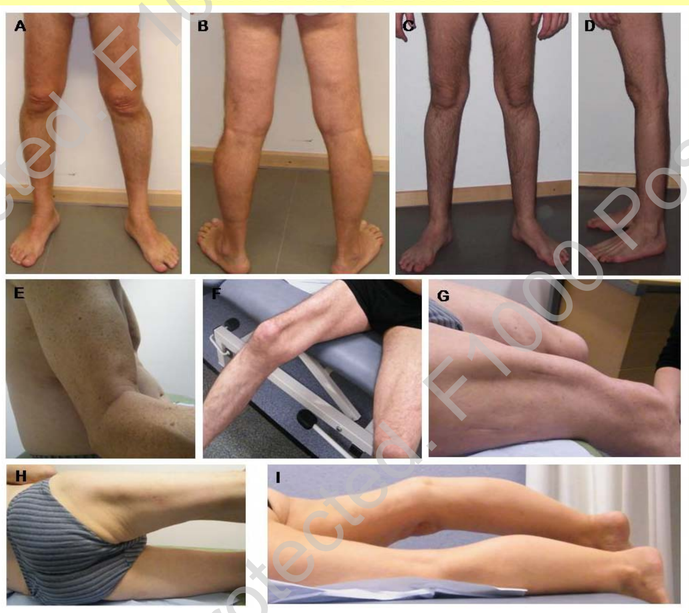
ANO5 sequencing and interpretative services are available in Newcastle on a commercial basis

Histological and immunoblot findings



(A), (B) and (C) H&E staining of muscle tissue from patients UK9, UK11 and UK5B showing myopathic or dystrophic changes. Notice the increase of internal nuclei (A), variation in fibre size, degeneration of muscle fibres (B) and increase in interfascicular and intrafascicular connective tissue (C). (D) and (E) neonatal myosin heavy chain labelling of muscle tissue from patient UK3 and UK11, respectively, indicating variable amounts of regenerating fibres. (F) Western Blot analysis for Dysferlin in patient UK9 showing a dysferlin band with normal size and amount.

Clinical assessment



(A) and (B) frontal and posterior view of the lower limbs of patient G3A showing atrophy of thighs and medial gastrocnemius and relative hypertrophy of lateral gastrocnemius. (C) and (D) frontal and lateral view of the lower limbs of patient G2 showing severe atrophy of quadriceps and calves. (E) Focal atrophy of biceps muscles of patient UK12. (F), (G) and (H) Severe hamstrings and quadriceps atrophy in patient UK3 and UK11. (I) Knee hyperextension in patient UK7A.

Pt.	Age (years)	Gender	Onset		CK (IU/L)	Pattern of Muscle involvement								other features
			decade	Symptoms		ambulant	UL prox	LL Prox	LL Distal	walk on toes	walk on heels	Muscle atrophy	scapular winging	AS	
UK1A	61	M	40s	walking difficulties	3500	yes	+/-	++	-	able	diff.	medial gastrocnemius	no	+	contractures (wrist, TA), SA, diabetes
UK1B	65*	M	40-50s	walking difficulties	4500	yes	-	++	-	N.A.	N.A.	-	no	-	diabetes, bladder cancer
UK2	50	M	20s	aches and pain	4000-8000	yes	+	+++	+	unable	diff.	quadriceps, hamstrings, gastrocnemius	no	+	myoglobinuria
UK3	45	M	20s	walking difficulties	4000-8000	yes	+++	+++	+/-	diff.	diff.	biceps, brachioradialis, quadriceps, hamstrings	yes	+	-
UK4	68	M	20s	walking difficulties	3000	restricted	+	+++	++	unable	diff.	deltoids, biceps, triceps, quadriceps	no	+	KH, foot drop, IHD
UK5A	37	M	20s	walking difficulties	5000	restricted	+/-	+++	-	able	diff.	medial gastrocnemius (AS)	yes	+	KH, contractures (wrist, fingers)
UK5B	43	M	20s	walking difficulties	5300	yes	+	+++	+	able	unable	brachioradialis, hamstrings, medial gastrocnemius	no	+	KH
UK6	61	M	40s	UL weakness	2400-3400	yes	+++	+++	+/-	able	able	biceps, brachioradialis, pectoralis, quadriceps, hamstrings (AS)	yes (AS)	+	KH, calf hypertrophy, contractures (wrist, fingers)
UK7A	54	F	20s	difficulties standing on toes	1800-10000	yes	-	++	+	unable	unable	quadriceps, medial gastrocnemius	yes	+	KH
UK7B	57	F	40s	difficulties standing on toes	3900	yes	-	+	+	unable	able	medial gastrocnemius	no	+	contractures (TA)
UK8	56	M	20s	difficulties standing on toes	2500	yes	-	+++	+	unable	able	biceps focally, glutei, quadriceps, hamstrings, medial gastrocnemius (AS)	no	+	KH
UK9	47	M	30s	calf wasting	4500	yes	-	+	++	unable	diff.	quadriceps, calves (AS)	no	+	-
UK10	55	M	30s	stiffness, knee problems	4100	yes	-	+++	+/-	able	diff.	Biceps focally, quadriceps, hamstrings, medial gastrocnemius (AS)	yes	+	KH
UK11	40	M	30s	walking difficulties	3000-7000	yes	+/-	+++	+	unable	able	quadriceps and calves (AS)	no	+	-
UK12	58	M	40s	walking difficulties	4400	yes	-	+++	+/-	unable	unable	biceps focally, thighs	no	+	-
G1A	49	M	30s	walking difficulties	2900-3500	severely restricted	+	+++	++	unable	unable	severe wasting LL muscles	yes (AS)	+	-
G1B	48	M	30s	↓ sport performance	800-4700	yes	+	+	+	able	diff.	quadriceps	no	-	calf hypertrophy, myoglobinuria
G2	35	M	late teens	↓ sport performance	5000	yes	+	++	+++	diff.	diff.	quadriceps, calves	no	+	-
G3A	58	M	40s	elevated CK	300-2000	yes	+	+	+	able	diff.	Medial gastrocnemius (AS)	no	+	-
G3B	56	M	30s	difficulties standing on toes	1700-3000	restricted	+	+++	++	unable	unable	quadriceps, calves	yes (AS)	+	myoglobinuria

Legend: Pt: patient number; CK: creatine kinase; UL: upper limbs; LL: lower limbs; AS: asymmetry; diff: able with difficulties; N.A: data not available; LGMD: limb girdle muscular dystrophy; DM: distal myopathy; RS: rigid spine; TA: Achilles tendons; SA: sleep apnoea; KH: knee hyperextension; IHD: ischemic heart disease; RRF: reduced respiratory function.* patient UK1B deceased at the age of 68 years of Bladder cancer. The patient was last seen in clinic at the age of 65 years.

- Clinical assessment defined the characteristics of LGMD2L in 20 patients
  - More than tenfold increased CK (20/20)
  - Proximal lower limb weakness (20/20)
  - Adult onset >20 years (19/20)
  - Muscle atrophy (19/20)
  - Asymmetry of muscle weakness/atrophy (18/20)
  - Distal lower limb weakness (17/20)
  - Upper limb proximal weakness (13/20)
  - Good sporting performance in presymptomatic period (8/20)
  - Knee hyperextension (7/20)
  - Scapular winging (6/20)
  - Restriction/loss of ambulation (4/20)
  - Contractures (4/20)
  - Myoglobinuria (3/20)

Conclusions

- The exon 5 c.191dupA mutation of ANO5 is a frequent cause of LGMD
- Patients with the common ANO5 mutation have a homogenous phenotype. The limited numbers of other mutations make conclusions tentative, but no clear genotype-phenotype correlations have emerged
- Mutations in ANO5 represent a common cause of adult onset muscular dystrophy with high CK and mutation screening, particularly of the common mutation c.191dupA, should be an early step in the diagnostic algorithm of patients fitting this clinical description