# Phenotypical and molecular definition of a cohort of 27 patients with Limb Girdle Muscular Dystrophy type 2A

Lazzarotto A, Bello L, Semplicini C, Fanin M, Sorarù G, Pegoraro E
Department of Neurosciences, University of Padova

**BACKGROUND.** We aimed to characterize the clinical features of a population of 27 patients affected with LGMD2A with validated, quantitative outcome measures (OMs), and correlate them with genetic and biochemical studies, in order to find reliable predictive parameters for progression during clinical follow up or future clinical trials. In our study we also collected plasma and serum samples for future experimental investigations on disease-specific biomarkers. We also evaluated retrospective data from 31 additional patients clinically followed in the past at the Neuromuscular Center of the University of Padova.

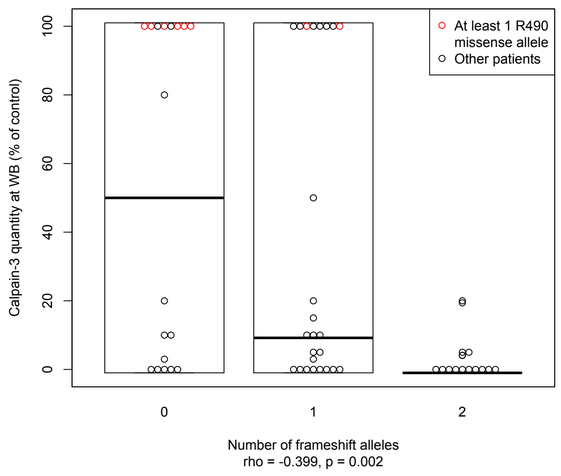


Image 1: Boxplot illustrating the correlation between calpain 3 quantity at Western Blot and number of frameshift alleles. Dots represent single patients. Red dots represent patients with mutation involving the amino acid in position p.R490.

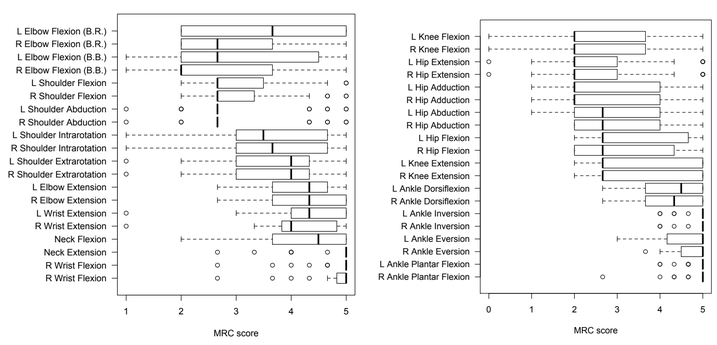


Image 2: Boxplots illustrating MRC scores for muscle groups (R, right; L, left). The MRC scale is indicated on the horizontal axis of the graphs. Upper panel: upper limb and trunk muscles. Lower panel: lower limb muscles. Muscles were sorted according to the median, in ascending order from top to the bottom of the Figure.

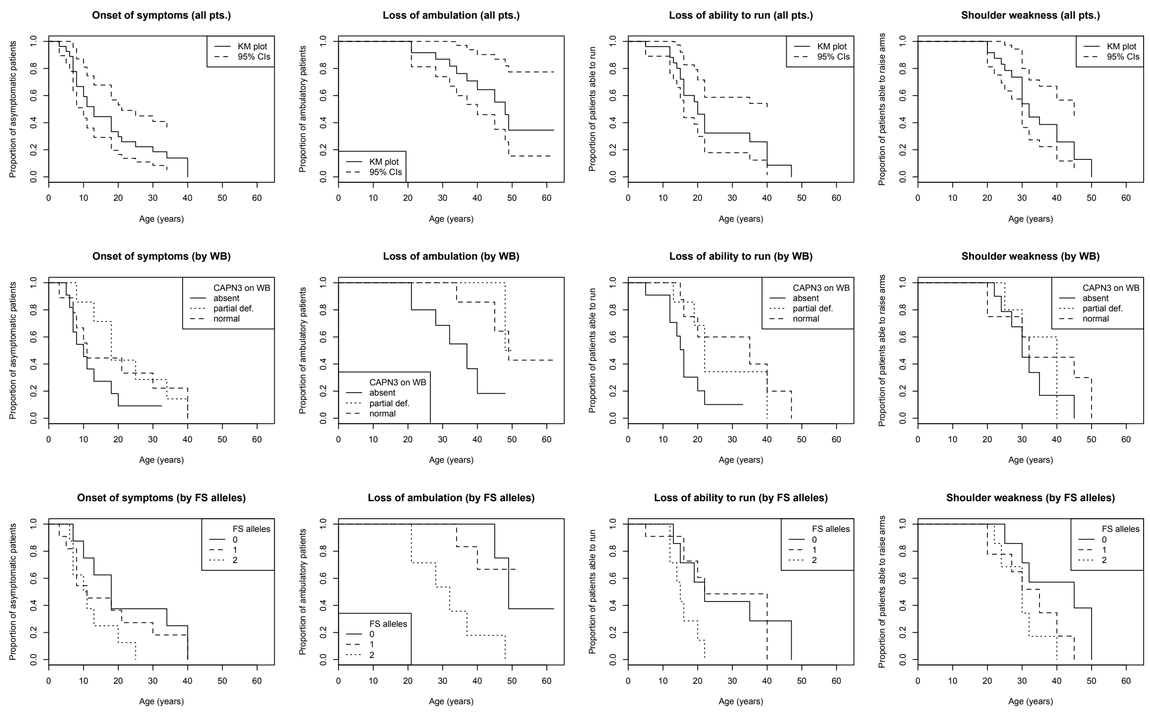


Image 3: Kaplan – Meier survival curve analysis for main disease milestones (onset of symptoms, loss of ambulation and ability to runm onset of disabling shoulder waekness) correlated with calpain 3 at Western Blot and frameshift alleles.

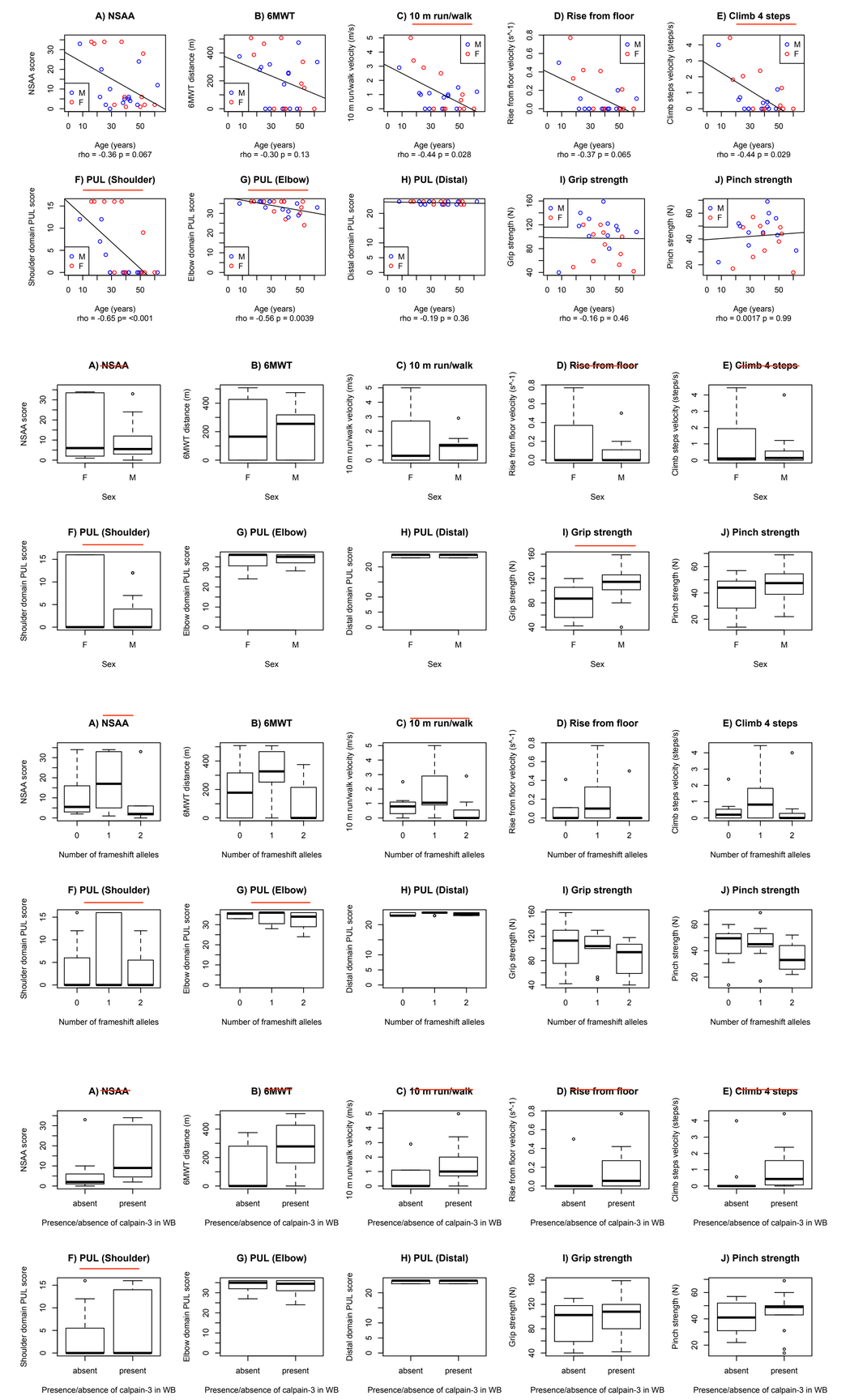


Image 4: Boxplots of evaluated OMs, correlated with age, sex, number of frameshift alleles and calpain 3 at Western Blot.

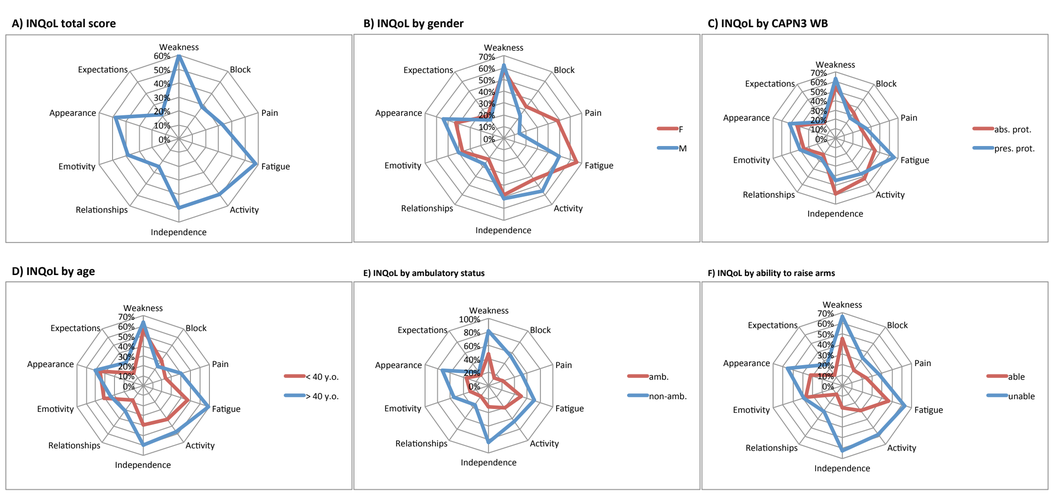


Image 5: radar charts of INQoL scores; total, and subdivided by gender, calpain 3 at Western Blot, age, and ability to walk and to raise arms.

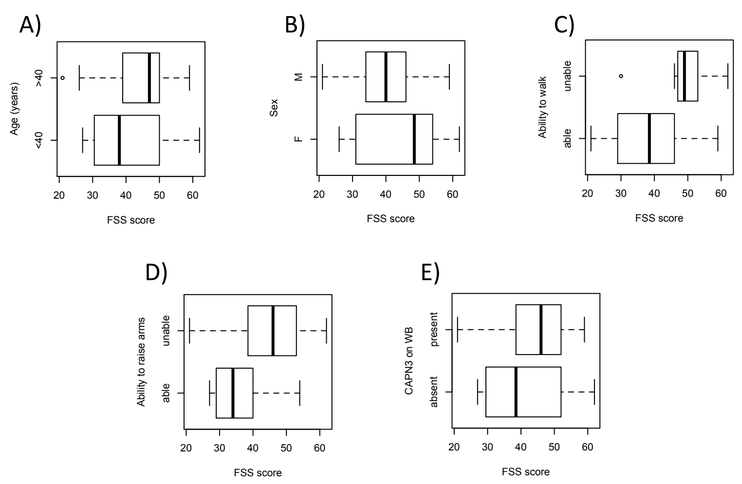


Image 6: Boxplots of FSS scores by age, sex, ability to walk and to raise arms, calpain 3 at Western Blot.

**RESULTS AND DISCUSSION.** Patients were divided by number of null alleles and by expression of protein at WB; these two variables showed a significant correlation. Complete calpain-3 deficiency at WB and number of null alleles were correlated with earlier age at disease milestones. **MMT** showed a specific pattern of weakness distribution, and a significant difference in strength between right (dominant) and left side in elbow flexor muscles. Absence of protein at WB and presence of 2 null alleles correlated with poorer results in tested outcome measures. Male patients showed significantly lower proximal PUL scores. Age showed moderate/strong correlations with NSAA, TFTs, and proximal/elbow domain PUL items, but not with 6MWT, distal PUL items, and grip/pinch strength. Protein amount by WB showed no linear correlation with OMs. There were strong intercorrelations between different OMs, excluding distal PUL and grip/pinch. INQoL showed significant worsening of the quality of life in non-ambulant patients and patients with shoulder weakness, and FSS scores showed an important impact of fatigue in LGMD2A. Cardiac and respiratory assessment resulted within normal limits. Sixteen of 58 patients (28%) showed a normal amount of calpain 3 but carried two pathogenic mutations in *CAPN3*. Most of these patients harboured, missense mutations in **exon 11**, and showed a peculiar clinical picture with relatively later onset and severe elbow flexion weakness. The presence of reduced or normal calpain-3 protein at WB, or of at least one non-frameshift allele, predicted a milder phenotype, suggesting that muscle pathology is alleviated when *CAPN3* mutations allow partial protein expression and function. **NSAA**, **6MWT**, **TFTs**, and shoulder/elbow **PUL** items appear feasible and clinically meaningful OMs for calpainopathy. **INQoL** and **FSS** are standardized, useful tools to assess how the diagnosis impacts on patients' daily life.

**CONCLUSIONS.** Our study suggests that in upcoming clinical trials for LGMD2A, patients should be stratified by genetic and/or biochemical criteria. Both functional OMs and patient-based questionnaires used in this study appear feasible endpoints for LGMD2A clinical trials. Future perspectives include longitudinal follow-up of these results.