8ª GIORNATA DELLO SPECIALIZZANDO IN NEUROLOGIA

Multiplex Ligation-Dependent Probe Amplification in undiagnosed autosomal recessive LGMDs

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LGMD2A
CAPN3

LGMD2B
DYSF

LIMB GIRDLE MUSCULAR DYSTROPHIES (LGMDs)

AUTOSOMAL RECESSIVE

- Sequencing of *CAPN3* and *DYSF* is laborious and time-consuming
  - Large size of coding sequence
  - Absence of mutational hot spots
- Although Next-Generation Sequencing (NGS) has improved the diagnostic rate of the molecular diagnosis in some LGMD2A/2B remains elusive

Define the frequency of deletion/duplication in a cohort of undiagnosed LGMD2A and LGMD2B cases studied through Multiplex ligation-dependent probe amplification (MLPA) technique

PATIENTS SELECTION

- Clinical suspect of
  - LGMD 2A (18 subjects)
  - LGMD2B (12 subjects)
- Sanger sequencing and NGS study: one or no candidate/causative mutations in coding genes
- Western blot (WB) analysis in muscle samples: protein reduction

MLPA ANALYSIS

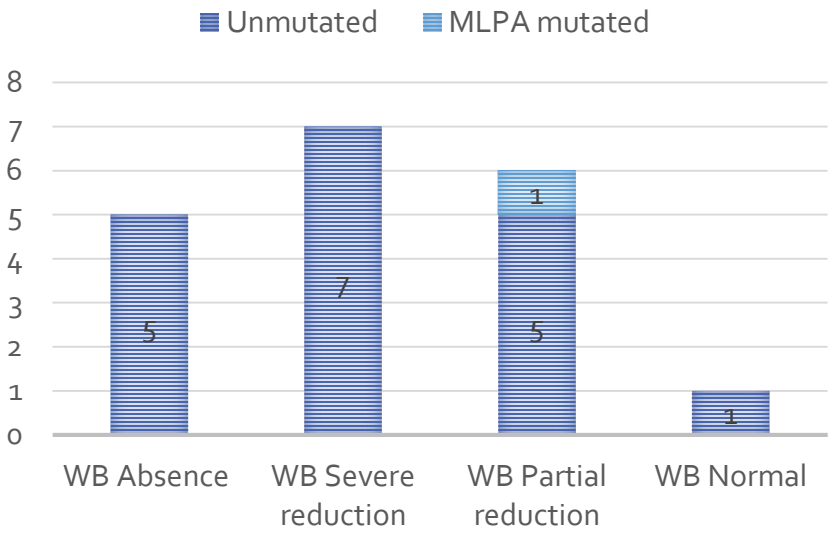
Probemix used:

- CAPN3 study: MLPA kit P176-C3 (MRC-Holland); probes for each of the 24 CAPN3 exons
- DYSF study: MLPA kit P268-A2 (MRC-Holland); probes for 40 different DYSF exons
  Exons not included: 3, 8, 11, 15, 19, 21, 26, 28, 32, 35, 38-39, 46, 48, 50

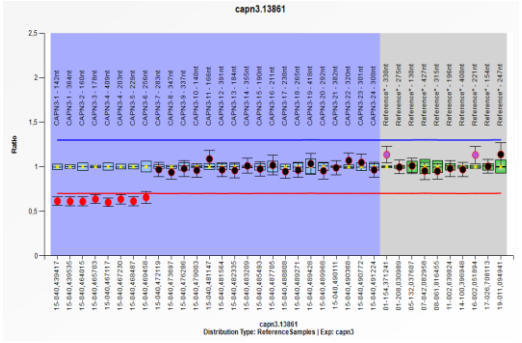
LGMD2A - *CAPN3*

18 patients → 1 mutated patient (5%)

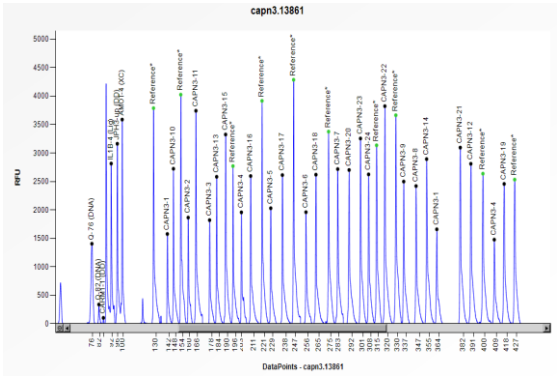
15 pts → 1 known mutation
3 pts → no known mutation



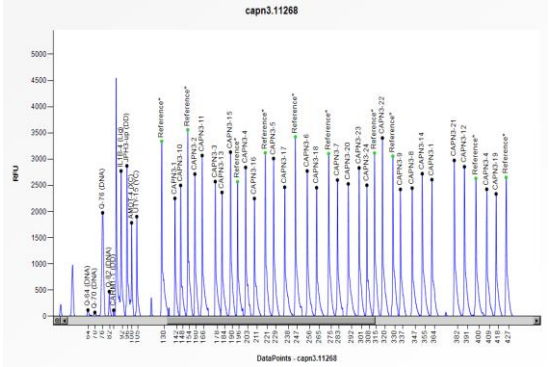
MLPA analysis



Patient I



Control

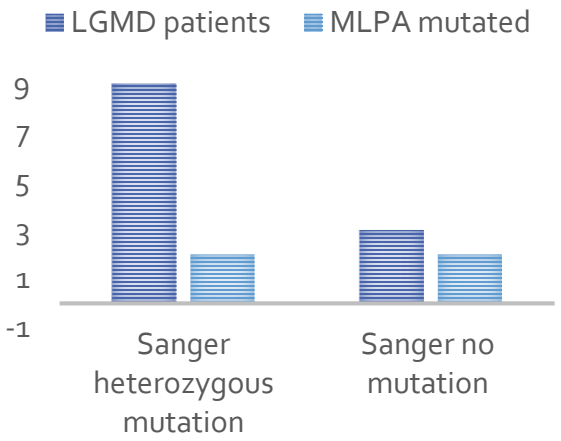


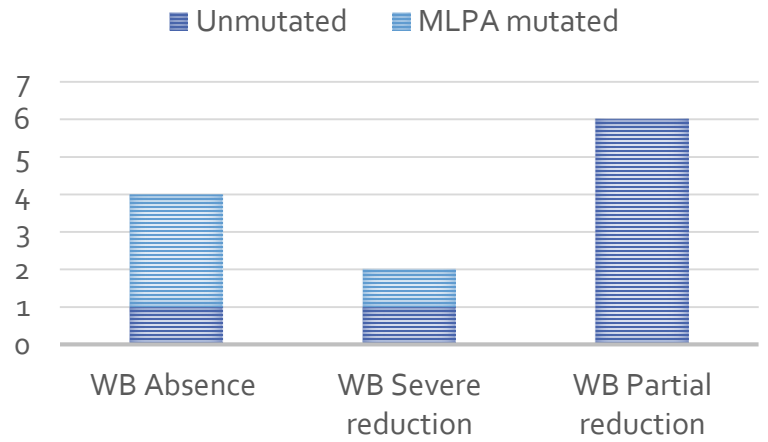
Patient I
Age: 46 year-old
Origin: Italian
Mutation:
c.1468C>T (Arg490Trp)
Deletion exon 1-6

RESULTS

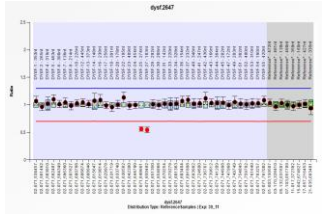
LGMD2B - *DYSF*

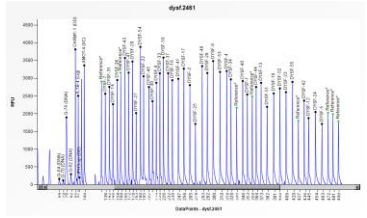
12 patients → 4 mutated patient (30%)



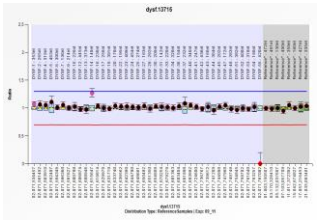


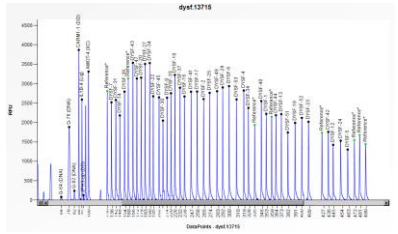
Patient I





Patient III





Patient I
Age : 41 years of age
Origin: German
WB: absence
Mutation:
c.1064_1065del (p.Lys355ArgfsX3) Deletion exon 25-27

Patient II
Age : 42 years of age
Origin: Italian
WB: severe reduction
Mutation:
c.2077delC (p.His693ThrfsX4) Deletion exon 25-27

Patient III
Age: 18 years of age
Origin: Egyptian
WB: absence
Mutation:
Homozygous deletion exon 55

Patient IV
Age: 40 years of age
Origin: Egyptian
WB: absence
Mutation:
Homozygous deletion exon 55

RESULTS

CONCLUSIONS

- MLPA analysis has been demonstrated to be useful in selected cases
- MLPA allowed a firm diagnosis in 30% of undiagnosed LGMD2B patients
- MLPA improved the molecular diagnosis in 5 of 26 patients (19%)
- It should not be used as a screening technique because it is tailored for the suspected candidate gene
- It is strongly suggested in cases with only one mutation identified and/or in case of protein absence at Western blot analysis