CLINICAL AND GENETIC HETEROGENEITY IN SIX PATIENTS WITH LIMB-GIRDLE MUSCULAR DYSTROPHY TYPE 2A

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BACKGROUND
Limbgirdle muscular dystrophies type 2A (LGMD2A) is the most frequent form of recessive LGMD and it is caused by mutation in the Calpain-3 (CAPN3) gene. LGMD2A is characterized by symmetric and progressive weakness of proximal muscles with onset ranging from two to 40 years. The mild to severe phenotype shows both intra- and interfamilial variability.

MATERIALS AND METHODS
We described six patients (aged 51-74) in four unrelated families. Each patient underwent a complete neurological examination and a muscle biopsy. In all cases morphological and immunohistochemical studies were performed. Mutation analyses included the coding exons of CAPN3 and their flanking intronic sequences.

RESULTS
All the patients presented limb-girdle muscle weakness. Despite the long course of the disease, all subjects were still ambulant at the time of our observation. Muscle biopsy showed severe dystrophic features in two and moderate myopathic changes in four cases. Pathogenic variants in CAPN3 were detected in six patients. In four patients, belonging from two different families, we found previously reported mutations. We could detect only the novel heterozygous c.526G>A in the proband of the third family. In that case, cDNA analysis did not disclose abnormal splicing, though we observed a strongly decreased CAPN3 mRNA, suggestive of a yet unidentified gene variant affecting mRNA stability or processing. Finally the sixth patient only carried the novel missense mutation c.2458T>C.

CONCLUSIONS
Here we describe clinical, histological and molecular findings in six patients carrying mutations in CAPN3, including two novel variants. Evidence for pathogenicity of the novel changes is highly suggestive for the following reasons: 1) they affected conserved amino acids 2) they were not present in 320 control chromosomes; 3) in silico tools predicted the novel missense mutations as being pathogenic. These data suggest that molecular analysis based on CAPN3 sequencing could not be considered conclusive for diagnostic purposes. In particular, in cases of heterozygosity the research of the second mutation should continue with the analysis of the transcript and possible gene rearrangements. Finally, this study confirms the genetic and phenotypic variability in patients with LGMD2A.