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Title of the Ph.D. Thesis: Calpainopathy: A clinical, histopathological and molecular study of Limb Girdle Muscular Dystrophy type 2A

Abstract

Limb girdle muscular dystrophies (LGMDs) are rare genetic autosomal recessive or dominant disorders mainly affecting the pelvic and shoulder-girdle muscles. The wide clinical and genetic variability of LGMDs make it difficult to achieve a precise diagnosis, especially in sporadic patients and requires a comprehensive clinical and laboratory approach. However in very few centers in India, diagnosis of about ten LGMDs is possible and status of rest of the entities is largely unknown due to lack of focus on molecular and genetic diagnosis. The purpose of this study was to subtype autosomal recessive limb girdle muscular dystrophies and ascertain their frequency. We used IHC, immunoblotting and gene sequencing to molecularly characterize our patients. Quantitative immunoblot analysis of calpain-3, dysferlin and sarcoglycans using immunoblot showed that LGMD2A constituting 60.8% of all LGMDs is most prevalent muscular dystrophy in north India. However 26.5% of the biopsies were characterized as LGMD2B, second most prevalent and 6.4% as LGMD-(C-F), probably third most prevalent/common form of LGMD in our cohort. 91.8% patients examined by sequencing had mutations in calpain-3 gene. Notably, we identified a total 90 novel and 26 previously reported mutations in our patients. Among all the variations, 74% were enriched in only eight exons of CAPN3. Since this is first genetic study of LGMD2A from India, we propose that in north Indian population the eight CAPN3 exons that are enriched with mutation in north Indian population could be analysed first for the genetic diagnosis of LGMD2A. Next we analyzed gene expression of NF-κB pathway related genes as this pathway has been hypothesized to play a key role in LGMD2A. We found a significant downregulation of M-line sarcomere marker, myom1 and associated genes myoD

and mef2c in LGMD2A indicating a possible regulatory link between these molecules and calpain-3. Moreover, we have also found that anti apoptotic gene XIAP and Ryr, a Ca^{++} ion channel receptor was downregulated in LGMD2A. Chromatin remodelers Brg1 that indirectly play a role in NFkB-IkBα mediated apoptotis regulatory mechanism and HDAC-4 & -7 were significantly downregulated in LGMD2A. The well established fact that HDAC interacts with NF-κB make it key molecule to further study the biology of muscular dystrophies. Notably we observed that UbB gene expression was highly downregulated in LGMD2A, which suggests that regulation of ubiquitin (Ub)–proteasome pathway may be under the control of calpain 3 activities. Overall, this study has successfully identified novel biomarkers that may be used to determine suitability of new treatments or therapies of muscular dystrophy.