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INTRONIC LONG INTERSPERSED NUCLEAR ELEMENT (LINE-1) INSERTION IN THE *DMD* GENE AS A CAUSE OF BECKER MUSCULAR DYSTROPHY

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Long interspersed nuclear elements (LINE-1 or L1) are the most abundant retrotransposable elements accounting for nearly 17% of the human genome. These elements can be randomly incorporated in the genome, therefore having an important role in its plasticity and in generating structural genetic variants. It has been demonstrated that L1 retrotransposon activity may occasionally cause genetic diseases. To date, only four disease-causing L1 elements have been described in the dystrophin (*DMD*) gene; three inserted in exons 44, 48 and 67, in patients with a Duchenne muscular dystrophy (DMD) phenotype, and one detected in the 5´untranslated region, in two apparently unrelated Japanese families with X-linked dilated cardiomyopathy.

We report a 48 year old man with a clinical diagnosis of Becker muscular dystrophy (BMD), in 2001, without molecular confirmation by multiplex PCR and Southern-Blot analysis, and whose diagnosis was recently revisited because his daughter is considering pregnancy. A second molecular study, resorting to multiplex ligation-probe amplification (MLPA) analysis and genomic *DMD* gene sequencing, again failed to detect abnormalities. A new muscle biopsy showed dystrophic features with irregular labeling for dystrophin on immunohistochemical analysis, suggesting dystrophinopathy.

With the intention of unveiling a genetic defect that might be refractory to the previous diagnostic techniques, muscle-derived *DMD* transcripts were sequenced in their entirety. Results revealed an insertion of 103 nucleotides between exons 51 and 52, which showed no homology to the gene's reference sequence. Extensive bioinformatic analysis (homology search and splice-site/branch-point analysis) and sequential direct sequencing enabled the discovery of a deep-intronic insertion of an L1 element, in intron 51. This extremely rare mutational event resulted in the partial exonization of the L1 plus 5 nucleotides of intron 51. In addition to the aberrant out-of-frame transcript, a residually expressed wild-type transcript was also detected, thereby explaining the milder phenotype in this patient.

To our knowledge this is the first report ever of dystrophinopathy caused by an intronically placed L1 element. Besides representing an exceptional contribution towards widening the *DMD* gene mutation spectrum, this study highlights the importance of conducting mRNA studies in as yet uncharacterized BMD/DMD patients. This holds true even considering some of the most recent state-of-the-art screening approaches, based on next-generation sequencing technology, where this type of mutation may ultimately fail to be detected.