Methylation profile of DMPK gene in myotonic dystrophy type 1 (DM1)

Myotonic dystrophy type 1 (DM1, Steinert's disease), is the most common muscular dystrophy in adult life, characterized by myotonia (prolonged muscle contractions), muscle weakness, cataract, cardiac disease, gastrointestinal abnormalities, and central nervous system dysfunction.

The clinical spectrum of DM1 patients is highly variable including congenital (cDM1), childhood onset cases with the most severe phenotype and the adult-onset.

The molecular defect underlying DM1 consists of an abnormal expansion of the cytosine-thymine-guanine (CTG) triplet in the 3' untranslated region (UTR) of the *DMPK* located on chromosome 19q13.2-q13.3. *DMPK* alleles up to 37 CTG repeats are considered normal. Individuals with CTG expansions in the pre-mutation range (from 38 to 49 repeats) have not been reported to have symptoms, but their children are at increased risk of inheriting a larger repeat size and thus having symptoms. Conversely, individuals with greater than 50 CTG repeats are almost invariably symptomatic.

The RNA transcribed from the expanded CTG repeat region forms intranucleoplasmic hairpin loops (foci) due to the extensive hydrogen bonding between C-G base pairs, and interferes with RNA-binding proteins, leading to decreased functional levels of muscleblind-like proteins (MBNLs) which are sequestered in the foci, and also increasing the steady-state levels of CELF1 (CUGBP1 and ETR-3-like factor) (Fig. 1A).

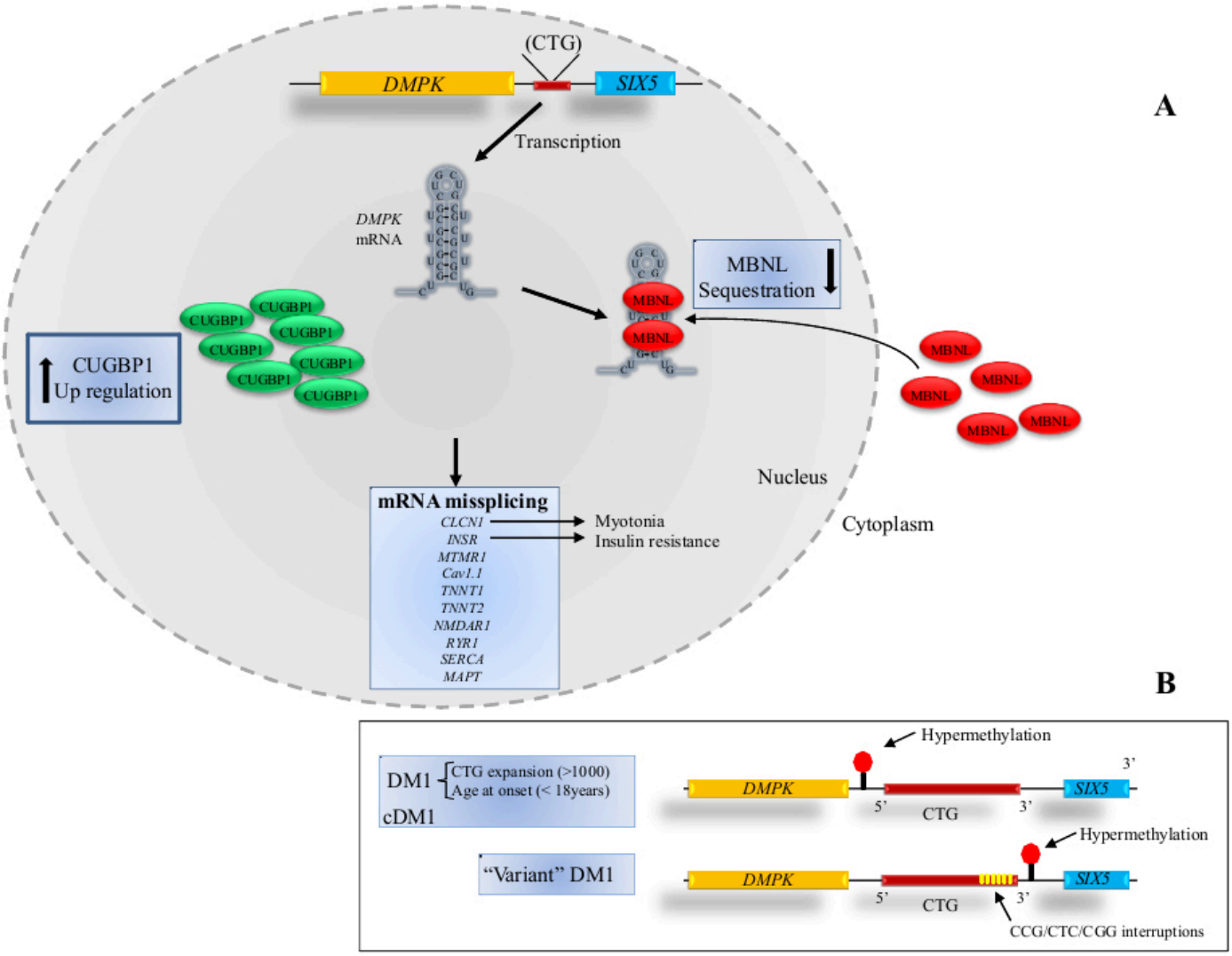

Fig.1. (A) Schematic representation of disease-related mechanisms of CUG-expansions in DM1. (B) Methylation distribution in DM1.

The main consequence of this toxic RNA function in DM1 tissues is a widespread splicing mysregulation of multiple genes ("spliceopathy") (Fig. 1A) in which embryonic isoforms are expressed in adult muscle, heart and brain with potential serious and life-threatening manifestations. In the past decade the DM1 locus has been assumed to contains a "pure" CTG repeat.

Recently, three independent studies documented that "variant" DM1 expanded alleles, containing unstable CCG/CTC/CGG sequence interruptions in the CTG array (3-5% of cases).

The “variant” repeats in the DM1 locus are strongly clustered at the 3’-end of the array revealing considerable polarity in the generation and/or maintenance of variant repeats. The introduction of multiple CUG, CCG and CGG sequences into the expanded array might change the methylation status of the region up and/or down of the repeat modulating chromatin structure the nucleosome assembly and therefore affecting the transcription levels and stability of the repeats.

In our study, DM1 uninterrupted alleles, hypermethylation occurs only in the upstream region of the CTG repeat, and this modification was present in patients with larger CTG expansion (>1000), earlier age at onset (

In “variants” DM1 patients with CCG/CTC/CGG interruptions, hypermethylation showed an opposite pattern compared to uninterrupted expanded alleles, occurring exclusively in the downstream region of the CTG array (Fig. 1B).

Since CAG repeats but not CGG repeats can stall the methyltransferase DNMT1, CGG/CTC/CCG repetitions interspersed into the CTG array of the “variant” DM1 alleles might interfere with insulation against methylation spreading to downstream regions exerted by the pure CTG stretch. The expanded repeat therefore demarcates an upstream boundary of methylation which could have a functional involvement in the transcriptional regulation of the entire 19q13.2-q13.3 region, including *SIX5* (gene downstream of *DMPK* locus) and the *DMPK* itself.

In our study, *SIX5* was even upregulated and *DMPK* was reduced in all DM1 patients, but these changes were independent from the methylation status of the DM1 allele.

In conclusion, age at onset, expansion size and presence of CCG/CTC/CGG sequence interruptions in the expanded CTG array are independently associated to hypermethylation at the *DMPK* locus in DM1. A better understanding of the precise cascade of processes induced by expanded trinucleotide repeats (TNRs) could provide therapeutic targets to alleviate disease progression and limit further TNR expansion.

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Publication

Expansion size and presence of CCG/CTC/CGG sequence interruptions in the expanded CTG array are independently associated to hypermethylation at the DMPK locus in myotonic dystrophy type 1 (DM1).
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