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Identification of a Reverted *DMPK* Allele and Analysis of Intergenerational Transmission in a DM1 Pedigree

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Abstract: Myotonic dystrophy type 1 (DM1) is an autosomal dominant, multisystemic disorder due to an unstable expansion of CTG repetition in the 3′ UTR of the *DMPK* gene, which increases in length during gametogenesis, causing genetic anticipation. Nevertheless, contraction of the DM1 expanded alleles has been reported in 10% of paternal and at 3% of maternal transmissions, mostly associated with less severe symptoms and a later age of onset. The aim of this study is to investigate the inheritance and meiotic instability of a paternal *DMPK* expanded allele that contracted into the normal range in an asymptomatic member of a DM1 family. We genetically characterized the *DMPK* gene in a DM1 family through a combination of SR-PCR, TP-PCR, LR-PCR and linkage analysis. We described a DM1 family with an asymptomatic 40-year-old woman inheriting from the affected father the *DMPK* repeat contracted in size to a $(CTG)_{30}$ allele. In prenatal diagnosis requested from the woman, we observed two *DMPK* alleles within the normal range in foetal DNA, one of them corresponding to the contracted maternal allele, which remained stable in the intergenerational transmission. We demonstrated, for the first time, that a reverted *DMPK* allele remains stable through maternal meiotic transmission, thus implementing our knowledge about the intergenerational dynamics of the DM1 locus.

Keywords: myotonic dystrophy type 1 (DM1); CTG repeat; *DMPK* gene; repeat contractions; meiotic transmission

1. Introduction

Myotonic dystrophy type 1 (DM1, MIM 160900) is an autosomal dominant multisystemic disorder characterized by progressive muscle weakness, myotonia, cognitive impairment, cataracts, cardiac arrhythmias, gastrointestinal and respiratory involvement, hypogonadism, and endocrine dysfunctions [1]. The phenotypic expression is extensively variable, ranging from asymptomatic adults to severely affected neonates with congenital onset of the disease [2]. Extreme clinical variability of the disease is partly explained by the genetic aetiology of DM1, caused by a CTG expansion in the 3′ untranslated region (3′ UTR) of the *dystrophia myotonica-protein kinase* (*DMPK*) gene located on chromosome 19q13 [3]. The number of CTG repeats in the *DMPK* gene is variable in the general population, usually ranging from 5 to 37 repeats [4]. DM1 patients have at least 50 CTG repeats in *DMPK* [4]. Individuals with a number of repeats between 38 and 49 are considered carriers of a "pre-mutation"

allele and can be asymptomatic throughout their lifetime, although they are at increased risk of having children with larger repeats [5]. In the last years, complex variant *DMPK* expanded alleles containing CAG, CCG, CTC and/or GGC interruptions have also been reported in about 3–10% of DM1 patients [6–8]. A hallmark of expanded pure CTG repeats is the instability during both mitotic and meiotic cell division, as demonstrated by intergenerational changes in the length of the repeat-containing DNA fragment of the *DMPK* gene [5]. This length typically increases in successive generations, resulting in an earlier onset and greater severity of the disease, a phenomenon known as genetic anticipation [9,10]. Moreover, penetrance and severity of disease tend to grow as repeat length increases, but extreme variability in penetrance end expressivity of specific symptoms exists in the patient population [11]. Nevertheless, anticipation is not an invariable occurrence and is modulated by the length of the repeat expansion as well as by the sex of the transmitting parent. In fact, DM1 pedigree analyses have demonstrated that approximately 90% of transmissions result in CTG repeat expansions, whereas about 10% lead to contractions [12]. The phenomenon of CTG repeat contraction in DM1 appears to be more frequently associated with paternal transmissions; however, the underlying mechanisms remain to be elucidated [13–15]. In rare instances, maternal contractions have also been reported in families with *DMPK* variant alleles harbouring CAG and CCG interruptions, which were additionally associated with low levels of somatic mosaicism in blood [12]. However, the majority of these cases describe a contraction in size remaining in the pathological range. Few cases are reported in which contraction leads to the formation of a normal reverted *DMPK* allele [16,17]. In these cases, carriers of the reverted allele are often asymptomatic at the age of onset of their affected parents. At the present time, no data are available about the behaviour of the contracted and reverted allele in the intergenerational transmission. Here we report the case of a DM1 pedigree in which a normal *DMPK* reverted allele, paternally inherited by an asymptomatic woman, remains stable through maternal transmission in the foetal DNA assessed by prenatal diagnosis. This study demonstrates that when a contraction occurs, the *DMPK* at-risk allele acquires stability even in maternal transmission, thus implementing knowledge about the intergenerational dynamics of the DM1 locus.

2. Materials and Methods

2.1. DM1 Family

This study was conducted on an Italian family with DM1. The family was comprised of 3 members, including two males and a female, and at a later stage, the pregnant woman's amniotic fluid was also acquired by our laboratory for molecular analysis of the *DMPK* gene. The enrolment of the subjects described in this study was approved by the Institutional Review Board of Policlinico Tor Vergata (Ethical Approval register numbers: 61/23). All individuals were referred to our centre for DM1 genetic testing, and informed consents have been obtained from each participant.

2.2. Molecular Characterisation of DM1 Patients

Genomic DNA was extracted from peripheral blood and a culture of amniocytes using the EZ1 Advanced XL Robotic workstation (QIAGEN, Germany). Molecular characterisation of the DM1 repeat expansion size was performed using a combination of Short-Range PCR (SR-PCR), bidirectional Triplet Primed-PCR (TP-PCR) and Long Range-PCR (LR-PCR), as previously described [8]. SR-PCR and TP-PCR products were separated by capillary electrophoresis on a 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) and analysed using GeneMapper™ 6 software (Thermo Fisher Scientific). LR-PCR fragments were subjected to agarose gel electrophoresis and capillary transfer to a nylon membrane. Subsequently, the membrane was hybridized with a CTG-repeated probe, which allows the measurement of (CTG)n repeats in the father, the brother, the proband and then for the amniocytes. Primers used are listed in Table S1.

2.3. STR Analysis

The DNA input for STR analysis was 0.5 ng. We used GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific) and 2720 Thermal Cycler (Thermo Fisher Scientific) to amplify 24 different loci. PCR products were separated by capillary electrophoresis on a 3500 Genetic Analyzer (Thermo Fisher Scientific) and analyzed using GeneMapper™ 6 software (Thermo Fisher Scientific).

2.4. Linkage Analysis

Linkage analysis, using two centromeric (D19S420, D19S903) and two telomeric (D19S412, D19S902) extragenic biallelic markers, was performed. 80 ng of DNA were amplified in a reaction volume of 15 µL, using True Allele PCR Premix master mix that contains AmpliTaq Gold DNA Polymerase, dNTPs, $MgCl_2$, and buffer (Thermo Fisher Scientific). Primers used are listed in Table S1. PCR conditions were the following: 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s and extension at 72 °C for 2 min. A final elongation was carried out at 72 °C for 10 min. PCR products were separated by capillary electrophoresis on a 3500 Genetic Analyzer (Thermo Fisher Scientific) and analyzed using GeneMapper™ 6 software (Thermo Fisher Scientific).

3. Results

3.1. Description of the DM1 Family

The family investigated in this study comprises a father affected by DM1 (I-1), his affected son (II-1), and an asymptomatic daughter (II-2). The proband's father died at the age of 65, with unknown DM1 symptoms onset and a progressive clinical decline reported from approximately 45 years of age. The proband's brother is also affected by DM1, with symptom onset in childhood followed by progressive clinical deterioration. He presents muscle weakness and wasting, ptosis, myotonia, diabetes mellitus, hepatic steatosis and mild right ventricle hypertrophy. II-2 is a 40-year-old woman requiring *DMPK* gene testing due to her positive family history, with the aim of assessing recurrence risk prior to conception. She has never had a neurological examination but reports being asymptomatic. Subsequently, she required DM1 prenatal analysis in her first pregnancy.

3.2. Combination of SR-PCR, TP-PCR and LR-PCR Methods for the Analysis of DMPK Alleles

We performed SR-PCR to characterize *DMPK* healthy range alleles in all family members (Figure 1). The woman's DNA (II-2) turned out to have two normal range alleles (5/30 CTG). The smaller allele (5 CTG) was inherited by II-2 from her mother (I-2) and it was shared with the affected brother (II-1). Surprisingly, the allele containing 30 CTG repeats didn't correspond with the normal range paternal allele (I-1), which appeared to have 7 CTG, suggesting a contraction of the paternal *DMPK* expanded allele to normal range. To confirm the SR-PCR results and exclude the possibility of somatic mosaicism, we analyzed DNA from a second sample of peripheral blood and from a buccal swab, obtaining identical results. DNA paternity test using 24 specific STRs indicates a 99.9% probability that I-1 is the biological father of II-2 (Figure S1). Simultaneously, we performed a bidirectional TP-PCR to detect DM1 expanded alleles in the family members (Figure 2). The father and son had a ladder pattern of electrophoretic profiles corresponding to an expansion of the *DMPK* gene at both the 3′ and 5′ ends of the CTG array. In contrast, the mother and the proband had a profile compatible with the presence of *DMPK* alleles in the normal range. We also performed a blotting of LR-PCR to estimate the size of the expanded *DMPK* alleles (Figure 3). This approach made it possible to confirm the presence of expanded *DMPK* alleles in the father and in the brother, assessing a CTG expansion of about 234–866 (class E2) and 487–750 (class E2) repeats, respectively. As we expected, no expansion was detectable in the woman's DNA or her unaffected mother. Successively, the proband required the prenatal molecular analysis of the *DMPK* gene on cultured amniocytes obtained via amniocentesis performed at 16 weeks of gestation. The foetal DNA revealed two alleles within the normal range, one of them corresponding to the reverted *DMPK* maternal allele of 30 CTG (Figure 1G) and the absence of CTG expansions by TP-PCR (Figure 2E) and blotting of LR-PCR (Figure 3).

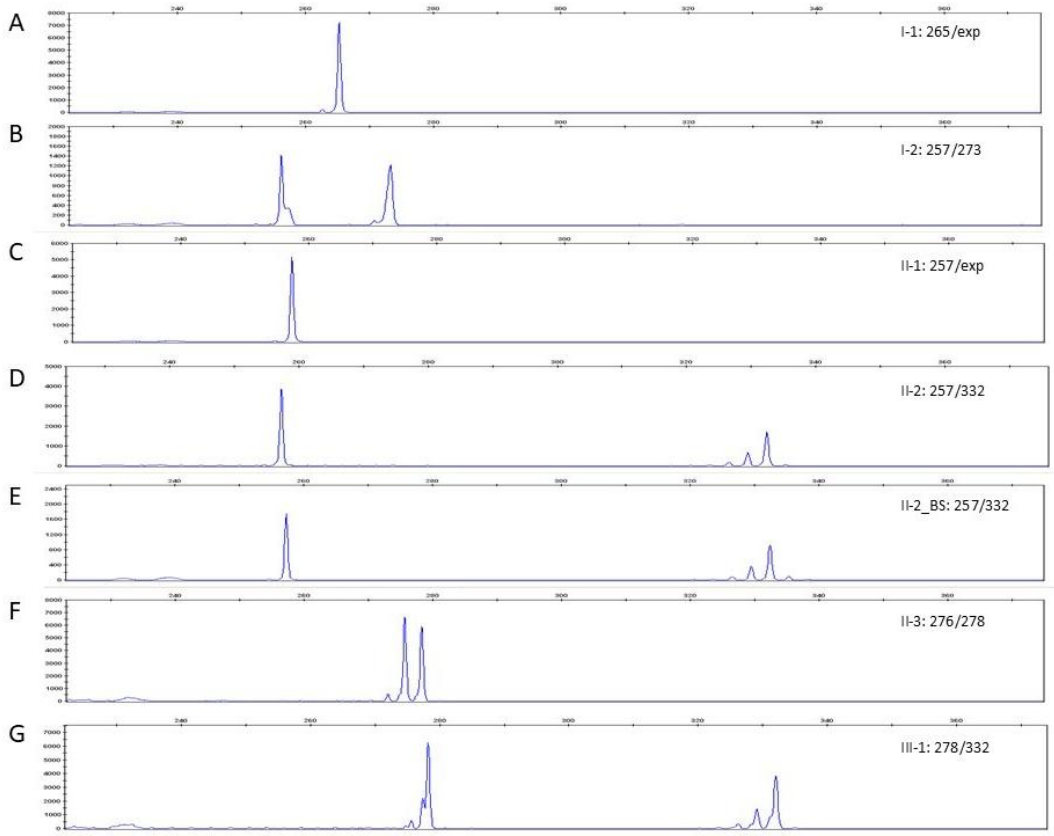

Figure 1. Electropherograms of SR-PCR products showing one normal range allele in the affected father's (**A**), and in the brother's samples (**C**). Two normal range alleles are instead viewable in the mother (**B**), in the proband's blood and buccal swab (**D**,**E**), in her husband and in the amniocyte's DNA (**F**,**G**). In each electropherogram, the analyzed subjects and the length of the SR-PCR products expressed in bp are reported.

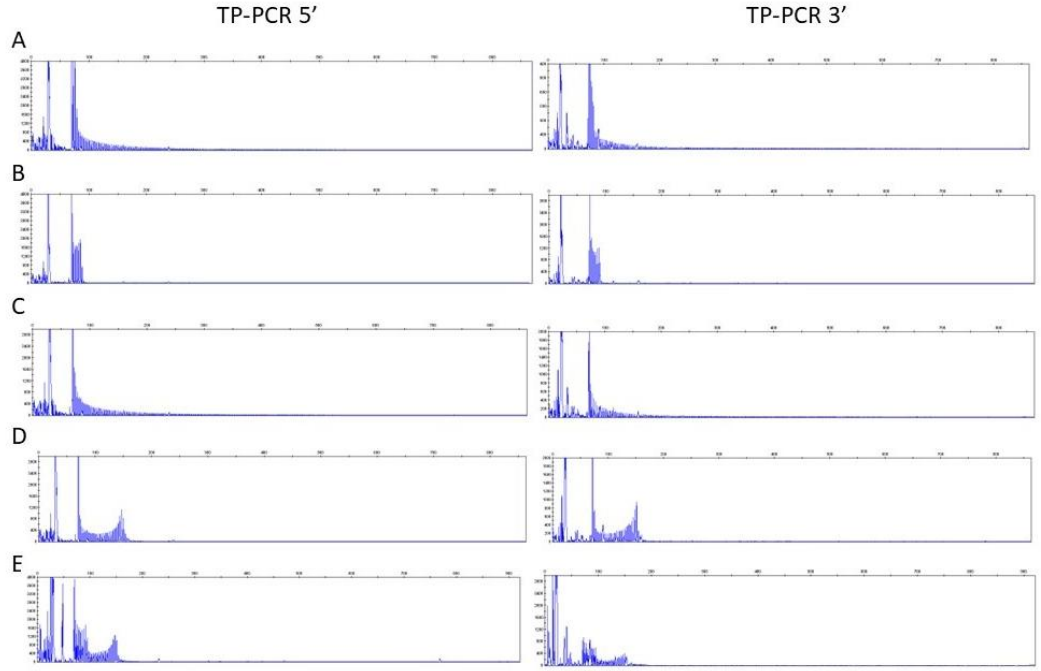

Figure 2. Electropherograms of TP-PCR profiles at 5′ and 3′ ends of the CTG array. (**A**,**C**): Father and brother present expanded profiles. (**B**,**D**,**E**): Mother, daughter and amniocytes show healthy range profiles.

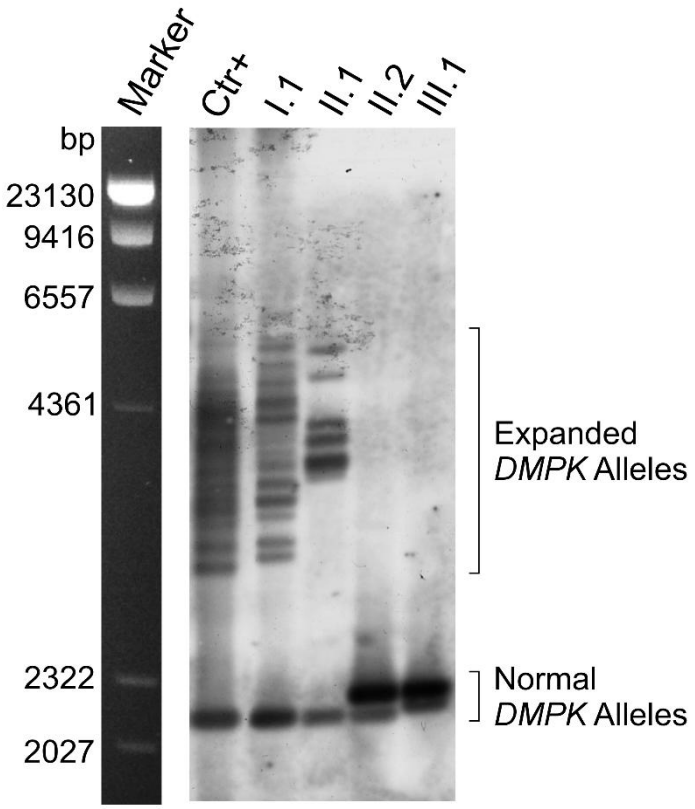

Figure 3. Blotting of LR-PCR products of the CTG repeat in the *DMPK* gene. I-1 and II-1: Father and brother present a pathological expanded profile other than the normal allele. II-2 and III-1: Proband and amniocytes show two alleles in the normal range. Marker: Lamba HindIII.

3.3. Linkage Analysis

In order to study the segregation of the DM1 alleles in our family, we also carried out indirect linkage analysis in all the family members. The analysis showed that the healthy proband, her affected father and brother, and the amniocytes shared the same at-risk haplotype to the *DMPK* locus (Figure 4). This result further confirmed the segregation of the familial at-risk DM1 allele in foetal DNA, showing that its contraction up to the *DMPK* normal range remains stable even in the maternal meiotic transmission.

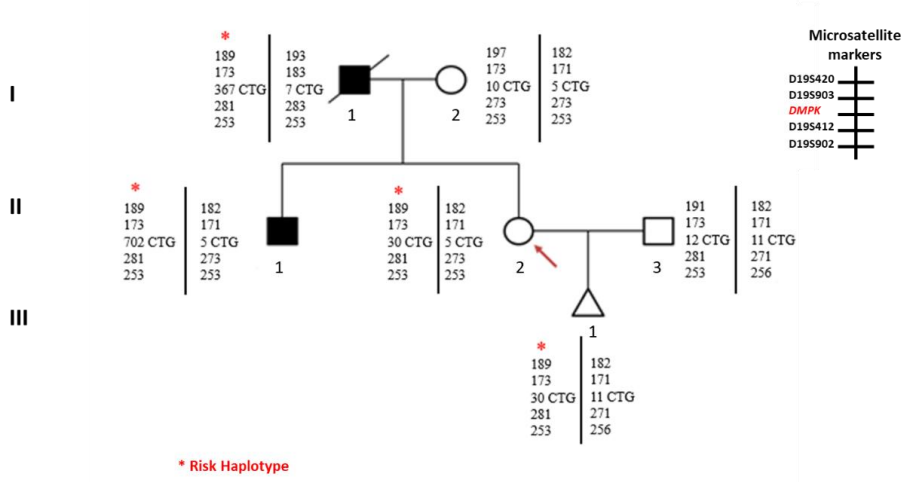

Figure 4. Pedigree of the DM1 family. Linkage analysis of four extragenic markers flanking the *DMPK* locus in the family shows the segregation of an at-risk haplotype in I-1 and II-1 DM1 affected members and in the healthy proband (II-2) and in the amniocytes sharing the reverted allele. Pathological and normal alleles in the *DMPK* gene are also shown. Open and solid symbols indicated unaffected and affected individuals, respectively. The red arrow indicates the proband; the red asterisk indicates the at-risk haplotype.

4. Discussion

CTG repeat expansions in DM1 are typically characterized by intergenerational instability, often resulting in further expansion, particularly in maternal transmissions, which are associated with a higher risk of congenital and juvenile-onset forms [18,19]. Conversely, contractions of expanded alleles, though rare, have been documented and are more commonly observed in paternal transmissions (10%), with reported contractions ranging from a few repeats to significant reductions bringing the allele into the premutation or normal range [16,17,20,21]. Maternal contractions have also been described, albeit less frequently [12,22]. The mechanisms underlying these contraction events remain poorly understood but are hypothesized to involve slipped-strand mispairing and aberrant repair during spermatogenesis [23]. Additionally, the meiotic behaviour of a contracted allele, particularly when it reverts fully into the normal range, is not well established, raising questions about the stability of these alleles in subsequent transmissions. The present study describes a family affected by DM1 in which a contraction of an expanded *DMPK* allele into the normal range was identified in an asymptomatic 40-year-old woman. Molecular analysis revealed that the woman had inherited from her affected father a contracted allele within the normal range, as confirmed by TP-PCR and LR-PCR results. As expected, her affected brother inherited an allele that underwent further expansion during paternal meiotic transmission. The results of prenatal testing requested by the proband demonstrated for the first time that once an expanded *DMPK* allele reverts to the normal range, its transmission does not re-expand during meiosis. We suppose that the reversion of the *DMPK* allele happens during the father's spermatogenesis. On the other hand, the proband is asymptomatic, suggesting that a post-zygotic reversion event is unlikely. This study has critical implications for genetic counselling, particularly in the context of preconception and prenatal risk assessments in families where a reversion event has occurred. This finding provides reassurance in counselling such families, suggesting that once a reversion to the normal range has been documented, the risk of re-expansion in the next generation is negligible.

Furthermore, our case underscores the necessity of a comprehensive family-based approach in genetic diagnostic practice, rather than focusing solely on the individual, particularly in asymptomatic subjects within DM1 pedigrees. This strategy allows for the identification of rare events such as allele contractions and ensures accurate risk assessment and appropriate reproductive counselling. It also highlights the relevance of incorporating extended family history and targeted molecular analyses into the evaluation of individuals from DM1 families to capture these exceptional yet clinically significant events.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/2509161445351292/IJND-25000033-Supplementary-materials.pdf. Figure S1: STRs analysis. Table S1: Primers used for SR-PCR, TP-PCR, and LR-PCR.

Author Contributions

Conceptualization, M.R.D.; Clinical evaluation, C.A. and E.P.; Genetic evaluation, V.V.V., P.B. and A.D.D.; writing—original draft preparation, V.V.V., P.B. and A.D.D.; writing—review and editing, M.R.D., A.B., G.N., F.S. and E.P.; supervision, M.R.D., A.B. and G.N.; project administration, G.N., funding acquisition, G.N. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of "Policlinico Tor Vergata" (Ethical Approval register numbers: 61/23).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article and its Supplementary Materials.

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Conflicts of Interest

The authors declare no conflict of interest.

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