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CASE REPORT

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Molecular characterization and reclassification of a 1.18 Mbp *DMD* duplication following positive carrier screening for Duchenne/Becker muscular dystrophy

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Abstract

A 2-month-old male patient harboring a duplication of *DMD* exons 1–7 classified as pathogenic by an outside institution presented with mildly elevated creatine phosphokinase (CK); molecular breakpoint analysis by our laboratory reclassified the duplication as likely benign. To date, proband continues to develop normally with decreased CK, further supporting our reclassification.

KEYWORDS

DMD, Duchenne muscular dystrophy, duplication, dystrophinopathy

1 | INTRODUCTION

Duchenne [DMD, MIM: 310200] and Becker [BMD, MIM: 300376] muscular dystrophies are X-linked disorders which result in progressive proximal muscle weakness and degeneration, in conjunction with characteristic elevation of creatine phosphokinase (CK) in blood. DMD is rapidly progressive and typically presents in early childhood with motor delays and gait instability, while BMD is characterized by a later-onset phenotype of skeletal muscle weakness.[1] Both DMD and BMD are caused by mutations in *DMD*, a ~ 2.2 megabase pair (Mbp) sized gene containing 79 exons encoding the dystrophin protein. Dystrophin maintains the structural integrity of striated muscle cells through the formation of a dystrophin-glycoprotein complex, linking the cytoskeleton to the extracellular matrix and thus providing tensile strength to muscle fibers.[2]

Given its size (roughly 0.1% of the human genome), a high degree of *DMD* allelic heterogeneity exists for DMD and BMD. Deletions are the most common pathogenic variants in *DMD* (~64% of cases), followed by nucleotide substitutions (22%), duplications (12%), and others (inversions, insertions ~2%).[3] These alterations disrupt dystrophin's reading frame in diverse ways, which can lead to mutated transcripts susceptible to nonsense mediated decay, truncated unstable proteins products targeted for degradation, or reduced activity protein variants.[4]

DMD duplications are up to five times underrepresented compared with deletions in public databases[3] and reported laboratory collections.[5] These variants have

historically posed a technical detection challenge as exonic duplications could not be captured by PCR-based techniques, fell outside interrogated probes by multiplex ligation-dependent probe amplification (MLPA), or were under the limit of detection of chromosome microarray (CMA). Such technical difficulties are coupled with the fact that duplications can adopt different structural configurations in the genome, including duplications in tandem direct or inverted orientations, or insertions into an entirely different chromosome, which can significantly alter the functional impact of duplications.[6] These limitations can challenge the clinical assessment of newly identified duplications in young asymptomatic individuals based solely on genomic data; for these individuals, further molecular and protein truncation characterizations need to be performed in order to provide an accurate diagnosis.

In this report, we illustrate the molecular characterization of a 1.18 Mbp *DMD* duplication spanning exons 1–7 in a 2-month-old male patient with mildly elevated CK levels. The duplication was found to expand up to 698 kilobase pair (Kbp) beyond *DMD*'s promoter regions and were positioned in direct tandem orientation relative to each other, which led to our reclassification of the variant as likely benign. Follow-up studies of proband at 13 months of age indicated decreasing CK levels and normal development, further supporting the likely benign reclassification of the duplication. We hope our study prompts further molecular breakpoint characterization of *DMD* duplications not only in young asymptomatic individuals but also in symptomatic patients, to further elucidate the impact of genomic duplications on *DMD* transcription and dystrophin structure and improve our interpretative capabilities for DMD and BMD diagnosis.

2 | RESULTS

2.1 | Case presentation

A 2-month-old White male patient (proband, IV-3 in Figure 1) was referred for genetic testing due to a duplication of *DMD* exons 1–7 identified in his mother (III-6 in Figure 1) during prenatal carrier screening by an outside laboratory; the duplication was classified as pathogenic by that laboratory. Proband was born at 39 weeks gestation by cesarean section; upon birth, he presented with jaundice, mild erythema toxicum neonatorum, and neonatal hypoglycemia that resolved with feeding. His CK levels at 2 months of age were 324 U/L. At 2 years of age, proband has a healthy normocephalic

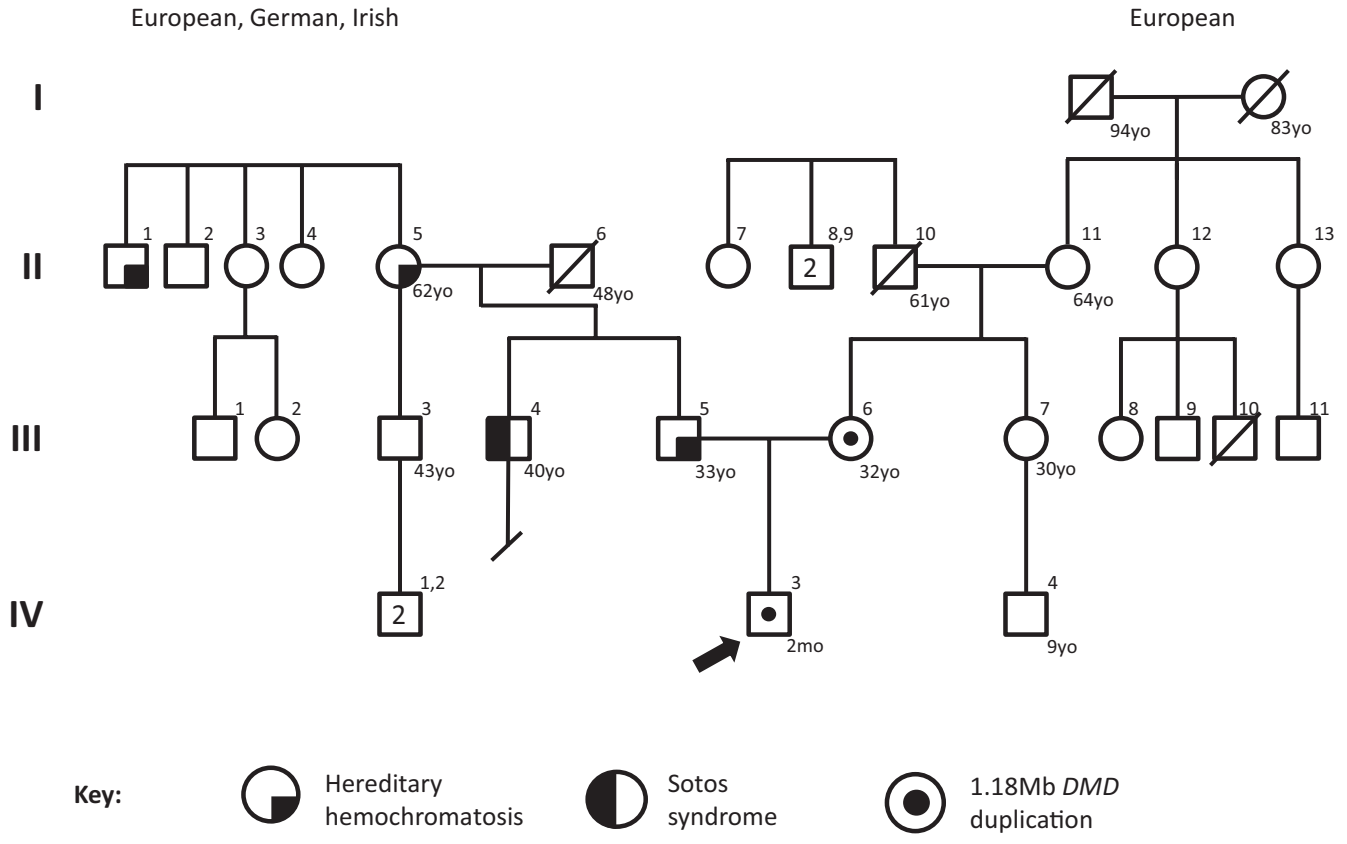

FIGURE 1 Pedigree showing the maternal segregation of a 1.18 Mbp duplication at Xp21.1 overlapping DMD. Proband is indicated with a black arrow (IV-2). There is no reported history of cardiomyopathy or dystrophinopathy in maternal and paternal families

appearance, is well-nourished, and well-developed; he has an active and alert mental status and normal mood, normal neck and chest shapes, normal motor strength and tone, normal gait, normal movement of all extremities, no contractures or tenderness, no calf hypertrophy, and CK levels of 211 U/L. His mother is a 33-year-old P1G1 White female patient with a history of infertility; she underwent clomiphene citrate treatment and intrauterine insemination for the currently reported pregnancy. Proband's mother also presented with gestational hypertension without evidence of preeclampsia during pregnancy and had normal electrocardiogram results after prenatal screening follow-up for potential cardiovascular disorders conferred by the detected *DMD* duplication. There is no family history of musculoskeletal concerns or cardiomyopathy for either of proband's parents.

2.2 | Genetic testing

Diagnostic MLPA testing was performed on proband to confirm the presence of the prenatally reported duplication, assess its size, and confirm a DMD diagnosis. MLPA experiments included probes targeting all *DMD* coding exons as well as its 5'UTR, 3'UTR, and alternative promoter/exon 1 DP427c, plus additional genomic control regions (MRC Holland). MLPA analysis was performed on ABI 3730xl (Applied Biosystems) and GeneMarker Software (SoftGenetics LLC, State College,). A duplication was identified spanning the 5'UTR to exon 7 of DMD (g.(?_33229574)_(32827735_32717219)) (hg19), with a minimal estimated size of ~513 Kbp. To fully define the size and location of the duplication, CMA analysis was performed on proband using CytoScan HD Suite (Thermo Fisher Scientific,). The duplication was found to be 1.18 Mbp in size, more than double the minimal size ascertained by MLPA, and overlapped the 5'UTR and exons 1–7 of *DMD* (NM_004006) plus additional upstream sequence (arr[hg19] Xp21.1(32,741,375- 33,926,846)x3 mat) (Figure S1). To further characterize the structural configuration of the duplication, CMA minimal and maximal probe positions were used to design sequencing primers (Primer3Plus), such that PCR products could distinguish between duplications in direct tandem orientation, inverted tandem orientation, or insertion elsewhere in the genome (Figure S2 and Supplemental Data). Using a long-range PCR protocol, forward primer ctgtgttttgggccatttct and reverse primer tgggtttagccctaggacac produced a ~ 1.8 Kbp band (Figure S3); this product was cleaned up using Exo-SAP-IT PCR Product Cleanup Reagent (ThermoFisher Scientific,) and Sanger sequenced on the ABI 3730xl (Applied Biosystems,). Chromatogram files were analyzed in FinchTV (Geospiza, Inc.) and FASTA files exported and mapped to hg19 using BLAT in the UCSC genome browser[7];. Sanger sequencing showed the duplication breakpoints to fall at Xp21.1:32,741,022 and Xp21.1:33,928,069 (hg19, Figure 2 and Figure S4). Breakpoint sequence analysis revealed the presence of short palindromic sequences at the junction as well as microhomologies in neighboring regions (Supplemental Data).

3 | DISCUSSION

We molecularly characterized a novel contiguous 1.18 Mbp duplication in *DMD* including its 5'UTR up to exon 7 in a two-month-old male patient with mildly elevated CK levels (324 U/L). The duplication was maternally inherited and initially discovered during prenatal carrier screening; the prenatal analysis reported this rearrangement to be a pathogenic exon 1–7 duplication, with the child being at a 50% risk of developing DMD. Follow-up diagnostic analyses of proband in our laboratory included MLPA, CMA, and Sanger sequencing; we showed this duplication to be in direct tandem orientation within Xp21.1, extending further away from exon 1 and including *DMD*'s 5'UTR as well as the DP427c alternative promoter/exon1 (Figure 2).

Sanger sequencing showed the duplication to encompass Xp21.1:32,741,022-33,928,069. There are ~570 Kbp of intervening sequence separating *DMD*'s DP427c isoform start (neuronal and retinal isoform)[8] and the duplication end, and ~ 698 Kbp separating the duplication end from Dp427m, which is the main isoform produced in muscle and is involved in DMD and BMD (Figure 2).[8] While we cannot rule out splicing defects in the normal *DMD* transcript caused by the presence of the duplicated *DMD* exons 1–7, the intervening ~570–698 Kbp of sequence between the duplication and the two main *DMD* promoters led us to hypothesize this variant to be likely benign, as no regulatory regions were separated from the main gene body, and any potentially truncated *DMD* product would likely undergo nonsense mediated decay (NMD). Our hypothesis was further supported by the observation that, at thirteen months of age, new measurements of CK levels in proband showed a reduction to 211 U/L (original measure was 324 U/L). In addition, proband remains asymptomatic and shows normal development without any noticeable neuromuscular issues or motor delays. Splicing analysis remains to be performed in future experiments, as we are currently unable to complete protein truncation testing (PTT) in proband due to the requirement of a muscle biopsy which is not recommended in an asymptomatic young child.[9] Family studies are being pursued, including

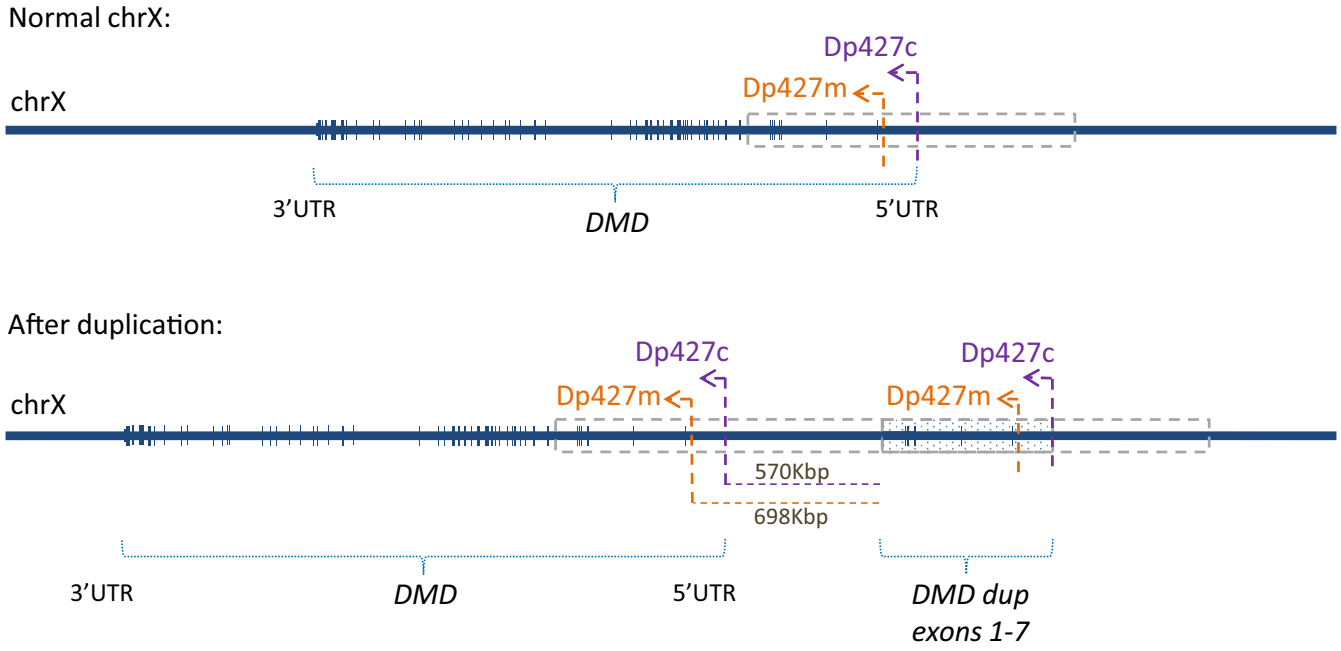

FIGURE 2 Schematic representation of *DMD* and the duplicated segment encompassing exons 1–7, 5'UTR, Dp427m, and Dp427c promoters. Notice the large intervening sequence separating the isoform promoters from the exon 7 in the duplication (dotted rectangle)

testing of males II-8, II-9, III-9, III-11, and IV-4 (Figure 1), to further clarify the significance of the duplication.

At the sequence level, the duplication described herein overlaps the duplication hotspot previously described for *DMD,* encompassing exons 2–20.[4] The majority of *DMD* duplications have different sizes and are non-recurrent events. These findings support non-homologous end-joining as a possible mechanism of generation. Junction analysis of the 1.18 Mbp duplication in proband revealed the presence of microhomology, which also suggests a possible origin through microhomology-mediated mechanisms such as fork stalling and template switching (FoSTeS)[10] and microhomology-mediated break-induced replication (MMBIR).[11] Microhomology tracts have been previously reported in the analysis of complex *DMD* rearrangements,[12] suggesting that several mechanisms can participate in DMD and BMD pathogenesis.

We surmise the exon 1–7 duplication was given a pathogenic classification by the prenatal screening laboratory based on a previous study that had reported an exon 1–7 duplication in an individual with the Duchenne phenotype.[13] Moreover, the mild CK elevation in proband could have prompted such overdiagnosis. Currently, most laboratories use a Gaussian distribution of 0–200 IU/L as the normal CK range in European-derived populations, with affected males having ranges >504 IU/L; however, it has been shown that CK levels in healthy populations can be skewed toward higher values, which can lead to overdiagnosis of mild abnormal levels.[14] This inconsistency may affect the diagnosis of asymptomatic infants with variants of uncertain significance (VUS) in *DMD* and mild CK elevations. In such instances, a comprehensive molecular characterization of the identified VUS, particularly duplications, is paramount to the correct prediction and diagnosis of dystrophinopathies. While the characterization performed in this study involved MLPA, CMA, and Sanger sequencing, clinical applications of next-generation sequencing (NGS) can, depending on the test design, provide breakpoint information and elucidation of duplication structure,[6] with the additional advantage of detecting exon point mutations and other smaller variants.[5] Of particular interest is the expanding use of long-read sequencing and optical mapping technologies for comprehensive human structural variant characterization.[15,16] As these techniques are more widely adopted in clinical laboratories, the identification of duplications with breakpoint in intronic sequences and more complex variants in *DMD* and other genes will be possible.

4 | CONCLUSION

Altogether, we have illustrated the importance of molecular characterization of *DMD* duplications in the clinical diagnosis of asymptomatic and young individuals. While duplications may escape finer descriptions due to current technical limitations, establishing their correct sizes and orientation is paramount to delivering an accurate clinical interpretation. As next-generation sequencing becomes more widely available in DMD

clinical testing, further molecular characterizations of duplication junctions in *DMD* can help elucidate and predict at-risk recombination sites, and better inform diagnostic capabilities.

AUTHOR CONTRIBUTIONS

CFF and JKGA provided clinical information. AM and CZM performed additional experiments for the molecular characterization of the DMD duplication. CZM, JEB, and LH wrote the manuscript. CZM, JEB, CFF, AM, JKGA, and LH read and approved the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article. Data is also available upon request to the corresponding author.

ETHICAL APPROVAL

Ethics approval for this study was received by the Mayo Clinic Institutional Review Board, application number 16–002365; participants were consented accordingly for release of data included in this study.

CONSENT

Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy.

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REFERENCES

1. Flanigan, K.M., Duchenne and Becker muscular dystrophies. *Neurol Clin*, 2014. 32(3): 671–688, viii.
2. Gao QQ, McNally EM. The dystrophin complex: structure, function, and implications for therapy. *Compr Physiol*. 2015;5(3):1223-1239.
3. Fokkema IF et al. *LOVD v.2.0: the next generation in gene variant databases*. *Hum Mutat*. 2011;32(5):557-563.
4. Aartsma-Rus A, van Deutekom JCT, Fokkema IF, van Ommen GJB, den Dunnen JT. Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. *Muscle Nerve*. 2006;34(2):135-144.
5. Zhang J, Ma D, Liu G, et al. Genetic analysis of 62 Chinese families with Duchenne muscular dystrophy and strategies of prenatal diagnosis in a single center. *BMC Med Genet*. 2019;20(1):180.
6. Newman S, Hermetz KE, Weckselblatt B, Rudd MK. Next-generation sequencing of duplication CNVs reveals that most are tandem and some create fusion genes at breakpoints. *Am J Hum Genet*. 2015;96(2):208-220.
7. Kuhn RM, Haussler D, Kent WJ. The UCSC genome browser and associated tools. *Brief Bioinform*. 2013;14(2):144-161.
8. Muntoni F, Torelli S, Ferlini A. Dystrophin and mutations: one gene, several proteins, multiple phenotypes. *Lancet Neurol*. 2003;2(12):731-740.
9. Aartsma-Rus A, Ginjaar IB, Bushby K. The importance of genetic diagnosis for Duchenne muscular dystrophy. *J Med Genet*. 2016;53(3):145-151.
10. Lee JA, Carvalho CM, Lupski JR. A DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders. *Cell*. 2007;131(7):1235-1247.
11. Hastings PJ, Ira G, Lupski JR. A microhomology-mediated break-induced replication model for the origin of human copy number variation. *PLoS Genet*. 2009;5(1):e1000327.
12. Baskin B, Stavropoulos DJ, Rebeiro PA, et al. Complex genomic rearrangements in the dystrophin gene due to replication-based mechanisms. *Mol Genet Genomic Med*. 2014;2(6):539-547.
13. Mah JK, Selby K, Campbell C, et al. A population-based study of dystrophin mutations in Canada. *Can J Neurol Sci*. 2011;38(3):465-474.
14. Moghadam-Kia S, Oddis CV, Aggarwal R. Approach to asymptomatic creatine kinase elevation. *Cleve Clin J Med*. 2016;83(1):37-42.
15. Chan S et al. Structural variation detection and analysis using Bionano optical mapping. *Methods Mol Biol*. 2018;1833:193-203.
16. Sedlazeck FJ, Lee H, Darby CA, Schatz MC. Piercing the dark matter: bioinformatics of long-range sequencing and mapping. *Nat Rev Genet*. 2018;19(6):329-346.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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