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Research Article

Identification of Mirror Repeats within mRNA Isoform (Cortical-dystrophin-Dp427c) of Duchenne Muscular Dystrophy Gene of Human Genome

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ABSTRACT

Duchenne Muscular Dystrophy (DMD) stands as the most prevalent and debilitating form of muscular dystrophy, caused by mutations in the dystrophin gene, which is recognized as the largest gene in the human genome. This X-linked recessive disorder is characterized by relentless skeletal muscle degeneration, leading to an early loss of mobility and tragically resulting in premature death due to respiratory or cardiac failure. The dystrophin gene spans approximately 2.2 Mb and produces multiple isoforms through distinct promoters and splicing events, exhibiting tissue-specific expression patterns. Despite advances in molecular diagnostics and understanding of dystrophin's structural role in maintaining sarcolemmal integrity, effective curative treatments remain unavailable. Recent studies in other neuromuscular disorders have demonstrated the pathogenic potential of repetitive DNA elements, particularly microsatellite expansions, which can disrupt gene function through transcriptional and post-transcriptional mechanisms. Among these, mirror repeats—symmetrical sequences can form non-canonical DNA structures—remain underexplored in relation to DMD. Considering the size and susceptibility to variations in the dystrophin gene, the presence of mirror repeats in its mRNA isoforms could have functional implications related to gene regulation, transcript stability, or mutational hotspots. This study aims to identify and characterized mirror repeats in full-length dystrophin mRNA isoforms to explore their potential role in the pathogenesis or modulation of DMD. The total of 284 mirror repeats is being identified in this study. DNA repeat expansions have been associated with more than 20 genetic neurological disorders. Understanding the contribution of such repeat elements might provide fresh perspectives on the molecular processes underlying dystrophinopathies, which can highlight novel avenues for biomarker development or therapeutic intervention.

Keywords: *Duchenne Muscular Dystrophy (DMD), Dystrophin Gene, Mirror Repeats, Neuromuscular Disorders, Gene Regulation.*

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INTRODUCTION

Human beings exhibit a remarkable diversity. Typically, this variation arises from genetic differences. Both genetic and environmental factors influence these differences. Substantial evidence indicates that genetic factors significantly contribute to human diseases. In technologically advanced countries, the rise of genetic disorders and congenital anomalies is becoming a significant concern that demands our immediate attention (Rasool & Shrivastav, 2002).

Mutations in the largest human gene, dystrophin, are strongly linked to significant health challenges and are pivotal in understanding various muscular dystrophies, varying from mild to severe forms. They include groups of genetic diseases that lead to progressive weakness and degeneration of muscles. More than 30 such disorders exist.

The reason behind these diseases is that inadequate or missing glycoproteins within the sarcolemma lies a crucial component that plays a vital role (Allen et al., 2016).

Table 1. Based on the difference in missing protein, Muscular dystrophies are of various types (Lovering et al., 2005)

DISEASE	DEFICIENT PROTEIN	AGE OF ONSET	MUSCLE AFFECTED
Duchenne (DMD)	Dystrophin	Early childhood	Muscles of the hip, legs, shoulders, spine, and the heart
Becker (BMD)	Dystrophin	Adolescence or adulthood	Similar to DMD
Limb-girdle (LGMD)	—	Adolescence to early adulthood	Proximal shoulder/pelvic girdle musculature
LGMD1A	Myotilin	Adolescence to early adulthood	Proximal shoulder/pelvic girdle musculature
LGMD1B	Lamin	Adolescence to early adulthood	Proximal shoulder/pelvic girdle musculature
LGMD1C	Caveolin-3	Adolescence to early adulthood	Proximal shoulder/pelvic girdle musculature
LGMD1D	Not identified	Adolescence to early adulthood	Proximal shoulder/pelvic girdle musculature
LGMD1E	Not identified	Adolescence to early adulthood	Proximal shoulder/pelvic girdle musculature

These conditions differ in terms of when they begin, the degree of severity, and the specific muscles that are impacted. Over time, all forms of MD become worse (Lovering et al., 2005). Duchenne muscular dystrophy (DMD) and Becker (BMD) muscular dystrophy are severe conditions that primarily affect skeletal muscle (Salari et al., 2002).

Muscular dystrophies are inherited disorders related to muscular tissue, marked by gradual muscle deterioration and weakness that can vary in pattern and intensity. In many cases of these conditions, the heart may be significantly impacted, even when there is minimal evidence of muscle weakness. The genes responsible for most of these disorders and the proteins they produce have been identified, which is crucial for accurate diagnosis, as well as effective genetic counselling and prenatal testing. At present, there is no proven method to change the long-term progression of these diseases effectively. This highlights the urgent need for innovative approaches in research and treatment (Emery & Allen, 2002). (DMD) is the most prevalent form of muscular dystrophy and also one of the debilitating forms, making its impact profound and significantly challenging for those affected. This condition poses significant challenges, affecting both physical strength and mobility from a young age, making it a critical area of focus. It is a disorder linked to the X chromosome, meaning the associated gene is located on this chromosome. It was initially detailed more than a hundred years ago. The deterioration of skeletal muscle marks DMD (McDonald et al., 1995; & Brooke et al., 1989).

Common causes of mortality in Duchenne Muscular Dystrophy (DMD)

include respiratory issues and heart failure due to the development of cardiomyopathy (Salari et al., 2002). DMD is a severe recessive genetic disorder linked to the X chromosome. The issue arises from a mutation in the DMD gene, which is responsible for encoding the dystrophin protein. Pathologically, DMD is characterized by the absence of this essential cytoskeletal protein. Clinically, it manifests as a progressive weakening of muscles, mainly affecting the proximal muscles in the limbs, neck, and chest. DMD is among the leading fatal neuromuscular conditions, affecting 1 in every 3,500 newborn boys. The clinical signs appear early in childhood, causing notable muscle wasting and weakness, which eventually leads to early death. Even though the protein defect exists from birth, clinical symptoms and diagnosis are not always present. These issues often emerge during the initial 2nd to 3rd critical development year of a child's life. As the disease progresses, individuals typically lose their ability to walk by around age 12, requiring the use of a wheelchair. Moreover, patients frequently develop severe scoliosis due to muscle weakness, with many confronting cardiac and respiratory collapse by their mid-twenties, especially if they do not seek ventilatory assistance (Limback et al., 2022; Wagner, 2002; Metule, 2002; & Biggar et al., 2001). The DMD gene is present on the Xp21.2 locus and encodes a rod-shaped structural protein found in the cytoplasm (Limback et al., 2022).

Duchenne Muscular Dystrophy occurs due to the lack of dystrophin protein. This 427 kDa protein is situated on the inner cytoplasmic side of the sarcolemma in both skeletal and cardiac muscle fibers. Dystrophin plays a crucial role in providing mechanical support to the plasma membrane of skeletal muscle. It facilitates the transfer of force between the extracellular matrix and the intracellular contractile machinery. Without dystrophin, the sarcolemma cannot endure the stress from regular muscle contractions. Without dystrophin, the sarcolemma cannot endure the stress from regular muscle contractions. This is due to the sarcolemma during muscle contractions (Petrof et al., 1993). Damage to the sarcolemma leads to an increase in calcium ions, which activate proteases, resulting in muscle fiber death (necrosis). As people get older, their capacity to regenerate muscle fibers diminishes, resulting in persistent muscle loss (Blau et al., 1983; Webster & Blau, 1990; & Bockhold et al., 1998).

Most individuals with Duchenne Muscular Dystrophy have normal intelligence, although some experience gentle cognitive impairments, with about 30 per cent scoring approximately 18 points below the average IQ (Bresolin et al., 1994). These individuals often struggle with memory, verbal learning, and attention deficits (Metules, 2002). The cognitive issues arise from changes in dystrophin isoforms displayed in the brain. (Culligan & Ohlendieck, 2002).

Various types of repetitive sequences have been identified in the genomes of different organisms (Mehrotra & Goyal, 2014; Biscotti et al., 2015; Dangi et al., 2024a; & Dhankhar et al., 2023a). These elements play significant roles in DNA repair, gene regulation, regulating the cell cycle, genome organization, determining chromosomal structure, evolution of karyotype, and chromosome rearrangements such as deletions, duplications, and inversions (Schimenti & Duncan, 1984; Shapiro & Von Stenberg, 2005; Yadav et al., 2022a; & Bhargava et al., 2023). A category of repeats that hasn't been thoroughly studied is mirror repeats. Mirror repeats denote a DNA or RNA sequence that is homologous to another segment within the same sequence. For instance, in the sequence 5'GAACCAACCAAG3', one segment (GAACCA) is homologous to another part of the same sequence (ACCAAG). These repetitions play a role in the development of non-canonical or non-B DNA configurations within the genome (Schroth & Ho, 1995). Research on myotonic dystrophy has shown that microsatellite repeats located in non-coding regions have the potential to be transcribed into pathogenic RNAs, highlighting a critical link between genetic

elements and disease mechanisms (Mankodi et al., 2000). The length of the repeats and their expression levels are correlated with disease severity and the age at which symptoms appear. However, variability across different tissues, somatic instability, and some technical challenges hinder the establishment of a perfect correlation and the identification of additional genetic factors that could either protect against or worsen specific disease symptoms (Kelvin et al., 2017). As of now, there have not been any studies focused on identifying mirror repeats in relation to Duchenne muscular dystrophy. As a result, this study aims to identify mirror repeats within mRNA isoforms associated with the muscular dystrophy gene, as this information could be beneficial.

The dystrophin protein gene stands out as one of the longest genes in the human genome, an impressive stretch of around 1.2 million base pairs, composed of 79 exons. This remarkable length highlights its complexity and significance in human biology (Hoffman, 1987). Due to its large size and intricate structure, the dystrophin gene is prone to a significant number of mutations. Approximately one-third of cases of Duchenne Muscular Dystrophy (DMD) occur due to new spontaneous mutations (Wagner, 2002; & Barbujani et al., 1885). The remaining cases typically exhibit an X-linked recessive inheritance pattern. Females who carry the mutation usually do not display symptoms of Duchenne muscular dystrophy because they have a second, healthy X chromosome inherited from their fathers. Generally, female carriers remain asymptomatic unless there is a problem with X chromosome inactivation or a chromosomal abnormality, both of which are pretty rare (Mathews, 2003).

The dystrophin gene, which is the longest gene in the human genome, measures 2.2 megabases in length and constitutes about 0.1 per cent of the overall human DNA sequence. It comprises 79 exons, 78 introns, and seven promoters, leading to the creation of seven isoforms that are meticulously regulated based on the tissue type. Various transcripts originating from the dystrophin gene yield multiple isoforms, three of which generate complete full-length mRNA (14 kb). The isoforms are designated as B for brain (Dp427b), M for muscle (Dp427m), and P for Purkinje (Dp427p). Each isoform includes distinct first exons combined with a standard set of 78 exons, demonstrating significant tissue specificity. Developmental processes regulate the production of full-length isoforms. The Purkinje isoform (Dp427p) is exclusively found in adult tissues. In contrast, the muscle isoform (Dp427m) and the brain isoform (Dp427b) can also be detected in human fetal skeletal and cardiac muscle (Torelli et al., 1999; & Panel Marie-Pierre Chevron et al., 1994).

Despite advancements in genetic diagnostics for muscular dystrophies (MDs), effective treatment options remain limited, focusing primarily on supportive care (Eagle, 2002). DNA repeat expansions have been associated with more than 20 genetic neurological disorders, such as Huntington's disorder and spinal muscular atrophy (La Spada & Taylor, 2010). In diseases caused by repeat expansions, the severity of the condition and age of onset are often related to the length of the repeats (La Spada, 1997). Studies on microsatellite repeats have also been carried out regarding myotonic dystrophy, showing that repeats located in non-coding regions can be transcribed into harmful RNAs (Mankodi et al., 2000). Discovering more genetic modifiers could assist in alleviating or exacerbating particular disease symptoms (Kevin et al., 2017). Therefore, studying repeats in the muscular dystrophy gene could be valuable.

MATERIALS AND METHODS

The nucleotide sequence of Cortical Dystrophin mRNA was obtained from the NCBI (National Center for Biotechnology Information) in FASTA format{Database version- NM_000109.4} (https://www.ncbi.nlm.nih.gov/). This entire nucleotide sequence was then divided into segments of 500 base pairs each. Each of these segments was treated as a query sequence, and

its reverse complement was generated using a reverse complement tool (https://www.bioinformatics.org/sms/revcomp.html). Both sequences were then aligned to check for similarities in local regions using the BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

In the findings, if the nucleotide sequences in both the subject & query were reversed, they identified a mirror repeat. A word size of 7 (for BLAST, it was 16) was used for the alignment, and the program parameters were set, with results recorded based on varying expected threshold values (E-value = 20). When performing BLAST, it's optimized for highly similar sequences (MEGABLAST). Mirror duplicates were identified by counting the maximum number of hits detected. When the particular position numbers in the subject & query sequences matched in reverse order, mirror repeats were established in alignments. The presence of a spacer between the subject and query sequences determined the classification of mirror repeats. The complete procedure is also represented in Figure 1.

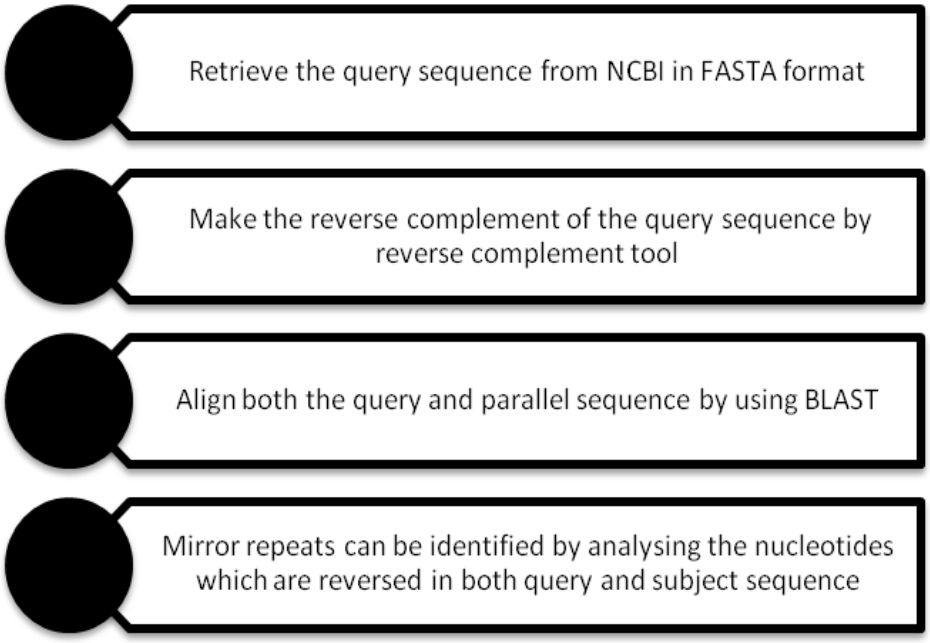

Figure 1: Process showing the Identification of Mirror repeats

RESULTS AND DISCUSSION

In this study, we employed a straightforward computational method to detect mirror repeats in the mRNA isoforms (Cortical-dystrophin-Dp427c) of the DMD gene in the human genome. The length of this mRNA is 14,069 base pairs, which were segmented into sections of 500 base pairs each to optimize the identification of mirror repeats. The highest number of findings was recorded with a threshold value of 20, leading to the identification of mirror repeats at this level. A total of 284 mirror repeats were discovered in this mRNA isoform. These mirror repeats were categorize into three types: Perfect Mirror Repeats (PMR), Perfect Mirror Repeats with Single Spacer (PMRSS), and Imperfect Mirror Repeats (IMR). Perfect mirror repeats feature identical sequences surrounding a central axis of symmetry, whereas imperfect mirror repeats contain mismatched sequences in the same context. A perfect mirror repeat may include zero or more spacer elements, depending on the spacing between them. According to Table 2, we identified 50 perfect mirror repeats, 41 imperfect mirror repeats, and 193 perfect mirror repeats with one spacer. The same data is represented in the form of a pie chart in Figure 2.

Table 2: Identified mirror repeats are as follows

S.No.	CDS LENGTH	EXPECTED THRESHOLD	NO. OF HITS	No.OF MIRROR REPEAT	MIRROR REPEATS	POSITION OF MIRROR REPEATS	TYPE AND FREQUENCY
1)	1-500	20	35	13	CAAAAGAAAAC AAATGGGTAAA AAAAGAAAA TGATTTAGT AGAAAGA CAGTGAC CAAAAAC GTAGATG AATATAA GAAAAAG AAGAGAA GTAAATG AATGTAA	148-158 166-176 288-296 366-374 41-47 222-228 277-283 397-403 436-442 292-298 135-141 172-178 463-469	Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1
2)	501-1000	20	37	7	TAATGTAAT ACCTATCCA AAGAAGAA CTGGGTC ACCACCA CCAGACC CTTCTTC	61-69 260-268 384-391 25-31 77-83 131-137 465-471	Perfect/1 Perfect/1 Perfect mirrorrepeat Perfect/1 Perfect/1 Perfect/1 Perfect/1
3)	1001-1500	20	35	11	GATGGGAATGCCTCAGGGTAG AAGTGGTGAA ACAAAAACA GGGCCGGG ACACACA GAAGAAG TCTTTCT TCATACT TACTCAT GTACATG AAACAAA	388-408 217-226 492-500 278-285 1-7 144-150 164-170 236-242 239-245 251-257 420-426	Imperfect/1 Perfect mirrorrepeat Perfect/1 Perfect mirrorrepeat Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1
4)	1501-2000	20	86	20	GAAGAAG GAACAAGAACAAG AAGAAGATCTAGAACAA ATGGTGGTGGTA TACAACAACAT GTAGTTGATG AAGAAAGAA AGAACAAGA AGAAAAAGA TCAAAAACT AAAAGAAAA AAAGAACAAGGAA TGGTGGTGGTAGT CAGAAGAC AGAAAAGA AACGAAAAAGAAA CTCACTC CACTCAC GTAGATG GAACAAG GATCTAG	144-150 100-112 89-105 136-147 68-78 145-154 2-10 99-107 327-335 399-407 431-439 5-17 137-149 239-246 429-436 432-444 127-133 129-135 230-236 345-351 424-430	Perfect/1 Perfect/1 Imperfect/1 Perfect mirrorrepeat Perfect/1 Perfect mirrorrepeat Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Imperfect/1 Imperfect/1 Perfect mirrorrepeat Perfect mirrorrepeat Imperfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1
5)	2001-2500	20	52	20	TCCACCACCACCT CGGAAGGAAGGC TCACCACCACT ACAAGGGAACA AGTTCAAAAACCTGA CCTCCACCACCACC ACTCGCTCA AAAAGAAAA AGAGCGAGA CAGAAGAC GGAAAAGG AGGTTGGA AGTCCTGA CAGTGAC GACCCAG ATCACTA ACAGACA	208-220 350-361 105-115 167-177 61-75 209-222 305-313 376-384 397-405 11-18 261-268 266-273 329-336 3-9 7-13 121-127 130-136	Perfect/1 Perfect/1 Perfect/1 Perfect/1 Imperfect Imperfect Perfect/1 Perfect/1 Perfect/1 Perfect mirrorrepeat Perfect mirrorrepeat Perfect mirrorrepeat Perfect mirrorrepeat

					TCAGACT GAAAAAG GAAAAAG	368-374 380-386 404-410	Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1
6)	2501-3000	20	44	10	AGTCTTTTCTGA GAAAGAGAAAG CTCCATACCTC CCCACCACCC TTAAAAATT TTAAAAATT GAGAAAGAG AAACCAAA AGAGAGA ACAGACA	359-370 290-300 485-495 168-177 207-215 267-275 384-392 475-482 381-387 395-401	Perfect mirrorrepeat Perfect/1 Perfect/1 Perfect mirrorrepeat Perfect/1 Perfect/1 Perfect/1 Perfect mirrorrepeat Perfect/1 Perfect/1
7)	3001-3500	20	36	6	CAGACTTTTAGTCAGTGATATTC AGAC AAGGAGGAA CTGGAAGAAGCTC GAGACTTGAGACAGAACTCAAAG CAGAGAC ACATACA	372-398 313-321 183-195 474-496 28-34 266-272	Imperfect Perfect/1 Imperfect Imperfect Perfect/1 Perfect/1
8)	3501-4000	20	35	15	GAGGTTTGGAG AAAGTGAAA AACCACCAA TCTAGAAAGACCT TTGAGAGAGTT TTGGAAGAAGTTT ATGAAGTA GAAGAAG AATATAA CTGAGTC TCCACCT AAGCGAA GTTATTG TCATACT AATTTAA	60-70 236-244 328-336 84-96 141-153 380-392 447-454 128-134 156-162 252-258 283-289 231-237 409-415 416-422 456-462	Perfect/1 Perfect/1 Perfect/1 Imperfect perfect/1 Imperfect Perfect mirrorrepeat Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1
9)	4001-4500	20	33	5	TTCACTT AACCCAA TTATATT AGGTGGA GACGCAG	20-26 54-60 284-290 298-304 303-309	Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1
11)	4501-5000	20	51	13	AAGTCTGAGTGAAGTGAAGTCTG AA GTGAAGTCTGAAGTG AAAAAGCAGACGGAAAA AAGTAAGATGATTTTAGATGAAG TGAA GAAGTGAAG GAAACAAAG GTGAAGTGAAGTCTGAAGTG GAAAGAAAG AAAGGAAA CTAAATC GAAAAAG AGAAAGA GGAAAGG	129-153 142-156 193-209 18-44 37-45 64-72 137-156 280-288 330-337 103-109 192-198 279-285 439-445	Imperfect Perfect/1 Imperfect Imperfect Perfect/1 Perfect/1 Imperfect Perfect/1 Perfect mirrorrepeat Perfect/1 Perfect/1 Perfect/1 Perfect/1
12)	5001-5500	20	41	7	CAGAATTAAGAC AGTCTTCTGA CAGAGAAAAAAGAAAC AGAAAAAGAAACCCCAGCAAAAA GA GGAGAGG AGGTGGA GGAAAGG	406-417 59-68 213-227 216-240 2-8 288-294 419-425	Perfect mirrorrepeat Perfect mirrorrepeat Imperfect Imperfect Perfect/1 Perfect/1 Perfect/1
13)	5501-6000	20	44	10	AGTCTTTTCTGA GAAAGAGAAAG CTCCATACCTC CCCACCACCC TTAAAAATT TTAAAAATT GAGAAAGAG AAACCAAA	359-370 290-300 485-495 168-177 207-215 267-275 384-392 475-482	Perfect mirrorrepeat Perfect/1 Perfect/1 Perfect mirrorrepeat Perfect/1 Perfect/1

					AGAGAGA ACAGACA	381-387 395-401	Perfect/1 Perfect mirrorrepeat Perfect/1 Perfect/1
14)	6001-6500	20	33	7	AGGAGTCTCTGAAGA CAGAAAGAC TCACACT AATATAA GGAAAGG CTCTCTC GAAAAAG	203-217 480-488 21-27 217-223 306-312 329-335 355-361	Imperfect Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1
15)	6501-7000	20	25	7	GAAAAGAGCAGCAACTAAAAGAA AAG ACTGTTGTCA AGAAAAAAAGA CAAAAAC CGGTGGC AGACAGA TTTGTTT	321-346 68-77 203-212 120-126 170-176 199-205 266-272	Imperfect Perfect mirrorrepeat Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1
16)	7001-7500	20	32	6	AACCAAACCAA AACCAACCAA TAGAAGAT TCTCCTACTCAGACTGTTACTCT AAATAAA CCTCTCC	166-176 162-171 307-314 408-430 55-61 406-412	Perfect/1 Perfect mirrorrepeat Perfect mirrorrepeat Imperfect Perfect/1 Perfect/1
17)	7501-8000	20	33	9	ACAAGAACA AAAAGAAAA ATGAAGTA GTGGGTG CGGAGGC AACACAA GAAGAAG GGACAGG GTAGATG	291-299 449-457 284-291 97-103 310-316 345-351 367-373 388-394 436-442	Perfect/1 Perfect/1 Perfect mirrorrepeat Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1
18)	8001-8500	20	41	11	CTTCAAGTGGAGTGAACTTC TGAACTTCAAGT CAGTTCCCCCTGGAC TGTAGATGT CCTGGAAGGTTCC AACTTCGGAAAAAGTCTCTCAA GTGAGTG GAGTGAG GAGCGAG GGAAAGG ACCTCCA	448-467 444-455 176-190 7-15 393-406 462-483 128-134 130-136 134-140 262-268 318-324	Imperfect Perfect mirrorrepeat Imperfect Perfect/1 Imperfect Imperfect Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1
19)	8501-9000	20	38	8	TCATGAGTACT CCGGGAACTTCAAGAGGCC ACGAAAGCA TGACCAGT GAATTAAG CGTCTGC GGTGTGG GAGAGAG	184-194 407-425 311-319 11-18 78-85 24-30 53-59 282-288	Perfect/1 Imperfect Perfect/1 Perfect mirrorrepeat Perfect mirrorrepeat Perfect/1 Perfect/1 Perfect/1
20)	9001-9500	20	36	7	AGGGTCCCTGGGA CATCTCGCCAAACAAAGTG CCCTAC CTTTCTTTC TGAAAGAGAACGT CTCTCTC GAGAGAG CAGGGAC	272-284 291-315 252-260 62-74 6-12 283-289 225-231	Perfect Imperfect Perfect/1 Imperfect Perfect/1 Perfect/1 Perfect/1
21)	9501-10000	20	19	0			
22)	10001-10500	20	23	5	GACTACATCAG AATGTAA AAACAAA AGGGGGA TACTCAT	185-195 34-40 230-236 304-310 395-401	Perfect/1 Perfect/1 Perfect/1 Perfect/1 Perfect/1
23)	10501-	20	31	15	AATCTGCAAGCAGAATATG	154-181	Imperfect

	11000				**ACCGTCTAA** **GTCCCCACTGCCGTCCCCT** **CCTG** **AAGTTTGAA** **GACTCCCCCCTGAG** **ACTGCGTCA** **TCCTCTCCT** **GAGGGGAG** **GAGTGAG** **AGAGAGA** **AGCACGA** **CCTGTCC** **CCTCTCC** **AACACAA** **AAGTGAA** **CGACAGC**	**207-229** **30-38** **43-56** **291-299** **433-441** **107-114** **96-102** **117-123** **188-194** **204-210** **242-248** **299-305** **413-419** **462-468**	**Imperfect** **Perfect/1** **Imperfect** **Perfect/1** **Perfect/1** **Perfect mirrorrepeat** **Perfect/1** **Perfect/1** **Perfect/1** **Perfect/1** **Perfect/1** **Perfect/1** **Perfect/1** **Perfect/1**
24)	**11001-11500**	**20**	**49**	**11**	**ATTGTTTTGTTA** **CTCAACAACTC** **TTATAATATT** **CAACTCAAC** **TGTTATTGT** **GATGAAGAAGGAG** **TCAAACT** **GAGAGAG** **AAATAAA** **TAACAAT** **TTTCTTT**	**393-404** **86-96** **276-285** **83-91** **389-397** **223-235** **1-7** **139-145** **324-330** **403-409** **494-500**	**perfect/1** **Perfect/1** **Perfect mirrorrepeat** **Perfect/1** **Perfect/1** **Imperfect** **Perfect/1** **Perfect/1** **Perfect/1** **Perfect/1** **Perfect/1**
25)	**11501-12000**	**20**	**42**	**10**	**GTTATAAAGAAAGATTG** **TTTAAAAATTT** **ACCACACCA** **TTGGGTT** **ACCACCA** **ACTGTCA** **TTGAGTT** **CTTTTTC** **AGAAAGA** **AAAGAAA**	**40-56** **23-33** **254-262** **15-21** **251-257** **292-298** **315-321** **404-410** **442-448** **444-450**	**Imperfect** **Perfect/1** **Perfect/1** **Perfect/1** **Perfect/1** **Perfect/1** **Perfect/1** **Perfect/1** **Perfect/1** **Perfect/1**
26)	**12001-12500**	**20**	**38**	**16**	**AGTAGCAGGACGATGA** **GTTGGTTGGTTG** **GTTGGTTGGTTGATTG** **ATTTAGATTTA** **GGTTGGTTGG** **AATACATAA** **ACACCACA** **ATTTTTTA** **CATAATAC** **CTCTTCTC** **AGGAGGA** **AAGGGAA** **GAGTGAG** **GATTTAG** **AAGTGAA** **ATTATTA**	**329-344** **180-191** **180-195** **242-252** **179-188** **429-437** **30-37** **311-318** **426-433** **471-478** **19-25** **127-133** **167-173** **241-247** **403-409** **460-466**	**Perfect mirrorrepeat** **Perfect mirrorepeat** **Imperfect** **Perfect/1** **Perfect mirrorrepeat** **Perfect/1** **Perfect mirrorrepeat** **Perfect mirrorrepeat** **Perfect mirrorrepeat** **Perfect mirrorrepeat** **Perfect/1** **Perfect mirrorrepeat** **Perfect/1** **Perfect/1** **Perfect/1** **Perfect/1**
27)	**12501-13000**	**20**	**34**	**8**	**TTTTTTAGATTTATT** **TTTAAAATAAATAAACTTT** **AAGAAAGAA** **TTTGGTTT** **GGAAAAGG** **AATTTAA** **ATAGATA**	**16-30** **71-89** **220-228** **1-8** **91-98** **69-75** **419-425**	**Imperfect** **Imperfect** **Perfect/1** **Perfect mirrorrepeat** **Perfect mirrorrepeat** **Perfect/1** **Perfect/1**
28)	**13001-13500**	**20**	**66**	**18**	**AATTAATTAATTAA** **TAATTAATTAAT** **ATTAATAATTA** **CAAAAGAAAAC** **TTCACCACTT** **ATTGCGTTA** **AAACCCAAA** **TTTGGTTT** **GGTTTTGG** **TTTGGTTT** **CCTTTTCTTTACC** **CATCCTAC** **CTAAAATC** **ATTATTA** **GTTTTTG** **ATTTTTA** **ATAAATA** **GAGTGAG**	**50-63** **49-60** **44-54** **215-225** **148-157** **162-170** **222-230** **100-107** **103-110** **266-273** **309-321** **365-372** **401-408** **41-47** **270-276** **327-333** **426-432** **479-485**	**Perfect mirrorrepeat** **Perfect mirrorrepeat** **Perfect/1** **Pemirror repeatect** **mirrorrepeat** **Perfect/1** **Perfect/1** **Perfect mirrorrepeat** **Perfect mirrorrepeat** **Perfect mirrorrepeat** **Imperfect** **Perfect mirrorrepeat** **Perfect mirrorrepeat** **Perfect/1** **Perfect/1** **Perfect/1** **Perfect/1** **Perfect/1**

29)	13501-13854	20	50	9	CATTATTTCGTGTTGTGTTC TTTATAAC TGAGTTCTTACTTGAGT TTATAACCACCAAGTATT TAATTAAT TGTTTTGT ATATTGTGTTTTA AATGTAA CATTTAC TCAGACT	52-79 160-176 73-90 39-46 136-143 185-197 105-111 213-219 265-271	Imperfect Imperfect Imperfect Perfect mirrorrepeat Perfect mirrorrepeat Imperfect Perfect/1 Perfect/1 Perfect

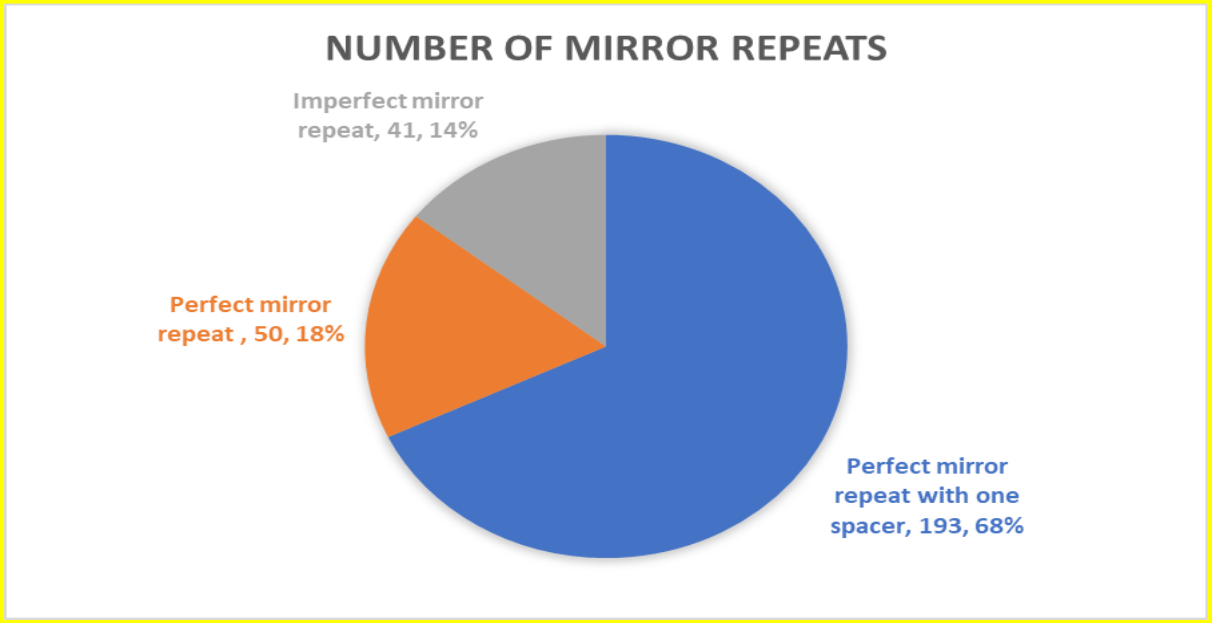

Figure 2: Chart showing the number of mirror repeats

CONCLUSION

In this study, we discovered that mRNA isoforms (Cortical-dystrophin-Dp427c) associated with the Duchenne muscular dystrophy gene are rich in mirror repeats. We identified a total of 284 mirror repeats across various regions using the BLAST tool. This mRNA comprises mirror repeats of different lengths and types. Understanding the significance of these mirror repeats at the molecular level could be crucial for comprehending muscular dystrophy. However, the precise role of mirror repeats has not yet been clarified. Additional research will be necessary to explore their exact function in this mRNA variant.

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There is no conflict of interest to declare.

Author Contribution:

All authors have participated in revising and approving the study design and analysis of the final manuscript.

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