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Analyses of the presence of mutations in Dystrophin protein to predict their relative influences in the onset of Duchenne Muscular Dystrophy

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ABSTRACT

Muscle plays a vital role in the life of vertebrates like humans. Muscle contraction is the only criterion required for locomotion. Muscle fibers also play a vital role as the provider of mechanical strength and act as a large repository of building blocks for protein synthesis in living beings. Muscles function as per the messages received from the extra-cellular signals. One of the central players responsible for capturing and transmission of extra-cellular signals to maintain the integrity of muscle function is the protein called Dystrophin (Dp). However, the wild type Dp protein accumulates some mutations which lead to a severe disease called Duchenne Muscular Dystrophy (DMD). The disease is so frequent that it is known to affect 1 in 3500 newborns per year. There are a number of reports that identify the mutations leading to DMD. Interestingly, it is also observed that the type of mutations affects the severity of the disease. But the biochemical mechanism of the DMD onset is still obscure. In the present scenario, an attempt has been made to analyze the mutations in the development of the disease. We analyzed the changes in secondary structure, solvent accessibility and stability of the Dp protein associated with the mutations. We tried to correlate the type of mutations with the severity of the disease. So far this is the first report that deals with the analyses of the mutations leading to DMD. This study would therefore be essential to come up with a plausible mechanism of DMD disease onset.

1. Introduction

1.1. Background

Human growth and development are very much dependent on the strength of muscle tissues, their masses and maintenance of proper integrity among them. Muscle plays a vital role as the provider of mechanical strength and acts as a large repository of building blocks for protein synthesis in living beings [1]. The main characteristic difference of muscle fibers from other tissue types is their ability to contract when needed. Muscles in association with other connective tissues carry out the movement of body parts as well as organs in living beings [2]. Among the three types of major muscle tissues, cardiac muscle maintains the activity of heart; smooth muscle tissue acts as the lining wall to vital organs; and skeletal muscle tissue confers us with the ability to move and provides the necessary mechanical strengths to perform regular day-to-day activities like walking [3]. It is therefore quite obvious that malfunctioning of muscular tissues will lead to disease conditions with varying degrees of severity. Muscle cell integrity is maintained by a number of factors. Notably among them is Dystrophin Associated Protein Complex (DAPC) which physically connects extra-cellular matrix proteins to cellular actin cytoskeleton and thereby maintains muscle cell membrane integrity and actin mediated muscle cell contraction [4]. The eminent members of this DAPC complex are Dystroglycan (DG), Sarcoglycans, and Dystrophin (Dp) in the order from their places of action from cell-membrane to cytosol. Sarcospan, Syntrophin, Dystrobrevin and other partner proteins also interact with Dp to maintain the cascade of flow of extra-cellular signals to the cell interior [4]. The protein DG serves as the main gateway for the transmission of the extra-cellular signals through the DAPC complex. DG has two subunits: alpha Dystroglycan (α-DG), the extra-cellular subunit which serves as the receptor of extra-cellular signals obtained through its extra-cellular partner ligand proteins and transmits the signal received from those partner proteins to the membrane bound subunit beta Dystroglycan (β-DG), the second subunit of DG. This β-DG with its C-terminal poly-proline rich region interacts with the WW domain of the cytosolic Dp protein which in turn interacts and regulates actin cytoskeleton with its N terminal Actin Binding Domain (ABD). The trans-membrane sub-complex of Sarcoglycan with its gamma subunit interacts with Dp protein whereas its delta subunit makes contact with DGs. In this way the stem of DAPC forms the bridge linking

Abbreviations: Dp, Dystrophin; DMD, Duchenne Muscular Dystrophy; DAPC, Dystrophin Associated Protein Complex; DG, Dystroglycan; MDDGC9, Muscular Dystrophy, Dystroglycanopathy, Type C9; OMIM, Online Mendelian Inheritance in Man; ABD, Actin Binding Domain; SpR, Spectrin Repeats; CRD, Cysteine Rich Domain; C-Term, C-terminal region; CH, Calponin Homology; ABS, Actin Binding Site; SASA, Solvent Accessible Surface Area; PDB, Protein Data Bank.

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extra-cellular matrix to cytosolic actin cytoskeleton [5]. A point mutation in α-DG, T192M, causes limb girdle muscular dystrophy, MDDGC9 [OMIM: 613818] which affects natural movement of individuals along with hampered cognitive inception and other symptoms [6]. While looking into the molecular details of disease generation, we have found that this single substitution of a polar amino acid by a non-polar amino acid (i.e., Threonine192 to Methionine192) has led to an increase in the hydrophobicity of the protein thereby decreasing its extent of interactions with its extra-cellular signaling partner, Laminin. This leads to the onset of disease limb girdle muscular dystrophy. Now this phenomenon itself exemplifies the effect of mutations in the system. On further analysis, it has been revealed that mutations in the members of the DAPC protein complex as well as the mutations in their partner proteins also cause different types of muscular dystrophies notably among them is the Duchenne Muscular Dystrophy (DMD), developed from the mutations in *DMD* gene that encodes Dp protein (427 kDa) [7]. Dp has four functional domains, namely, Actin Binding Domain, Spectrin Repeats, Cysteine Rich Domain and C-terminal. Mutations in the functional regions of the Dp protein lead to onset of muscular disorders with varying degrees of severity. However, among the different types of muscular dystrophies, DMD is the most severe one. DMD, an X-linked disorder, is the most common muscular dystrophy worldwide affecting 1 in 3500 newborns yearly [8]. It has a high rate of morbidity and mortality, with a frequency of 20,000 deaths annually at early childhood. The large size of the *DMD gene* makes it susceptible toward mutations [7]. The mutations in the DMD gene and thereby in the Dp protein not only causes DMD but also is associated with less severe form Becker Muscular Dystrophy and X-linked Cardiomyopathy [9]. However, the severity and the type of muscular dystrophy are dependent on the nature of mutation and the region of occurrence of the mutations in the Dp protein. The types of mutations leading to DMD have been categorized as: deletions, duplication and point mutations. It has been found that the major contribution to the DMD disease comes from deletion types of mutations followed by duplication, both leading to premature termination of translation of the *DMD* gene [10]. On the other hand, point mutations in the *DMD* gene lead to formation of mutated protein products causing DMD. Although rigorous works have been done to identify the mutations leading to DMD, the biochemical mechanism behind the onset of the DMD disease due to the point mutations in the Dp protein is still not clear. In the present scenario, we made an attempt to analyze the point mutations in Dp protein and tried to correlate the effects of the mutations with disease onset. For collection of the mutation data we used information from patients suffering from DMD as mentioned in different databases. We categorized the mutations as pathogenic or not and analyzed the probable structural changes in the Dp protein associated with these mutations. Our analysis identified the possible roles of the mutations in the onset of the DMD disease. We also tried to establish the relationship between type of mutations and severity of the disease. This is so far the first bioinformatic report that analyzes the correlations between appearance of mutations and their corresponding effects on disease development. This work would therefore be beneficial to explore the molecular details of DMD onset.

2. Materials and methods

2.1. Filtering mutations linked with DMD consequence

The amino acid sequences of the proteins involved in muscular dystrophy have been extracted to collect the causative DMD mutations. The mutations have been isolated from three different databases:

1. UMD-DMD (http://www.umd.be/DMD/W_DMD/index.html): This database was built in a joined national effort involving different diagnostic laboratories in France to provide up-to-date information about mutations in the *DMD* gene identified in patients with dystrophinopathies.
2. DMD database for Utah (http://www.genome.utah.edu/DMD/): This database provides us with information about the new mutations. The database collects information from a number of different subjects. The data from subjects participating in the "Utah Dystrophinopathy Project" have detailed historical and phenotypic information. The database also gathers information from samples received from other physicians not directly participating in the "Utah Dystrophinopathy Project". The phenotypes of these patients are also listed in the reports as received by the physicians [11].
3. e-Dystrophin (http://www.cureduchenne.org/edystrophy.html): This database is basically a database reporting all variants of human Dp produced by in-frame *DMD* gene mutations. We searched the database thoroughly and selected only those mutations that are designated as mis-sense mutations coding for a different amino acid in Dp protein and have a DMD phenotype.

Some mutations in the record from 'DMD database for Utah' showed phenotype B/DMD. Those were also taken into account. A total of 18 unique mutations have been collected which are known to cause DMD phenotype in affected individuals. Table 1 presents a detailed list of the mutations collected for our analysis.

2.2. Processing of sequence to study the effects of the mutations

To analyze the effects of the mutations, a stretch of amino acids was extracted from the original sequence of full length Dp protein (UniprotKB P11532) from human. We then incorporated the necessary mutations in the amino acid sequence of the wild type Dp protein one by one to generate the mutant proteins. To consider the effect of the mutation amino acid sequences were considered using a 201 amino acid window as per [12]. For example if position X is the position of interest i.e., amino acid sequence position in the Dp protein where mutation occurred in patients suffering from DMD, then the sequence stretch considered for our study would be X-100 to X + 100. For each type of mutation two sets i.e. wild type set and mutant set were prepared. The final dataset contained a total of 36 sets of sequences.

2.3. Analysis of the effect of mutations

To assess the effects of the mutations on the structure and function of the Dp protein we analyzed the following features of the wild type and mutant Dp proteins:

a) The secondary structures of the protein before and after mutations: For analyzing secondary structure of the wild type and mutant Dp proteins PSI-PRED [13], SPPIDER [http://sppider.cchmc.org/sppider_doc.html] and CFSSP web servers [14] were used. We used a number of different servers in order to get a consensus result [15].This method is like a cross-validation method. If the mutated residue was found to reside at turn positions then the type of the turn (whether type I or Type II turn [16]) was further detected with NetTurnP server [17].
b) The stability of the protein before and after the mutation: The stabilities of the Dp proteins before and after mutation were analyzed with Mupro server [http://www.ics.uci.edu/~baldig/mutation.html].
c) Protein hydropathy profile: This was checked with PROTPARAM [http://web.expasy.org/protparam/] server that employs Kyte Dolittle algorithm for assigning hydrophobicity score to each amino acid. The effects of mutations on the variations of solvent accessibilities from the amino acid sequences of the wild type and mutant Dp proteins were detected by SPPIDER and DSSP software [18].
d) The severity of the mutation: This was checked with Mutation assessor [http://mutationassessor.org/], PON-P [19], SIFT [20], Poly Phen2 [20], SNAP [21], SNPs&GO servers [http://snps-and-go.biocomp.unibo.it/snps-and-go/] to generate a consensus conclusion. Amino acid sequences of the wild type and mutant Dp proteins were further given as an input to NCBI-Conserved domain search (http://www.