RESEARCH ARTICLE

Analysis of the conformational changes caused by the mutations in mitofusin2 gene by Insilico approach

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Abstract

Objective: To find the effect of pathogenic Mitofusin 2 mutations, responsible for Charcot-Marie-Tooth hereditary neuropathy type 2A, on protein structure.

Methods: The study was conducted at department of biosciences COMSATS University Islamabad, Sahiwal campus from September 2016 to July 2017, and comprised patients with Charcot-Marie-Tooth hereditary neuropathy type 2A who were divided into early-onset severe group A and late-onset mild group B. Bioinformatics and molecular analysis was done to find the changes in the protein structure caused by the mutation. Three mutations were selected in two domains of the gene. These were: p. Arg94Trp, p. His165Arg and p. Thr362Met.

Results: Of the 10 patients, 5(50%) were in each of the two groups. Change in the structure was predicted in the mutated protein at position p. Arg94Trp, and, due to the mutation, an extra alpha helix was formed in the mutated protein.

Conclusion: Change in the structure of protein can be in a critical position that is involved in the mitochondrial fusion process. However, further studies are required to validate and explain the findings.

Keywords: Inherited peripheral neuropathies, CMT2A, MFN2, Functional disability scale, Structural analysis, GTPase domain.

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Introduction

Inherited Peripheral Neuropathies (IPNs) are the most common and heterogeneous form of motor and sensory disorders[1] Charcot-Marie-Tooth (CMT) disease is one of the most familiar forms of IPN. CMT disease is commonly separated into two types CMT1, or the demyelinating type, and CMT2, the axonal form. Further classification is made on a genetic basis.[2] Currently more than 80 genes are responsible for the CMT disorder. The locus of Mitofusin2 (MFN2) gene is 1p36.22 which encodes a mitochondrial membrane protein that participates in mitochondrial fusion and contributes to the maintenance and operation of the mitochondrial network. This protein is involved in the regulation of vascular smooth muscle cell proliferation, and it may play a role in the pathophysiology of obesity. Mitochondrial dynamics refers to the continuous change in size, shape and position of mitochondria within cells. Abnormalities of mitochondrial dynamics produced by mutations in proteins involved in mitochondrial fusion MFN2, fission ganglioside-induced differentiation-associated protein-1 (GDAP1), and mitochondrial axonal transport usually present with a CMT phenotype. MFN2 mutations cause CMT2A by altering mitochondrial fusion and trafficking along the axonal microtubule system.[3] CMT2A is mainly originated by mutation in the MFN2 gene.[4] MFN2 mutations are the frequent cause of CMT disease.[5] Various pathogenic MFN2 mutations showed two categories of phenotypes according to disease severity and onset age.[6,7] There were 2 main groups of patients, including those with early onset aged <10 years and those with late onset aged >10 years.[8] Patients with early onset showed severe symptoms with associated symptoms of scoliosis and contractures, while late onset had milder symptoms.[9-11] MFN2 gene provides instruction for making the MFN2 protein which decides the share[12] and structure of mitochondria. Being a dynamic structure, mitochondria goes through processes called fusion and fission to perform proper functioning.[13] The fusion process is controlled by MFN2 protein.[10,14] Membrane transport between storage space in eukaryotic cells demands proteins that let the budding and scission of emergent cargo vesicles from one compartment and their targeting and fusion with another.[15]

The guanosine triphosphate-ase (GTPase) region is the most extremely conserved domain as analysed with the Ras superfamily, while dynamin has an unusually high GTPase activity and low affinity for GTP. Thus, it has a low basal GTPase activity which is controlled by self-assembly or lipid binding. Correct arrangement of axonal mitochondria is critical for multiple neuronal activities. To understand the underlying mechanisms for population behaviour, quantitative characterisation of elemental dynamics on multiple time scales is required.[16] GTPase domain is thought to be strongly involved in mitochondrial

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fusion and in hydrolysis of GTP. Mutations in GTPase domain are responsible for binding and hydrolysis of GTP and can disrupt the fusion process which ultimately leads to various disorders.[17] The current study was planned to find the effect of mutations in MFN2 gene in different domains on the structure of protein.

Patients and Methods

The study was conducted at department of biosciences COMSATS University Islamabad, Sahiwal campus from September 2016to July 2017and comprised CMT2A patients who were divided into early-onset severe group A and late-onset mild group B. Severity of the disease was described one the basis of functional disability scale[18] (FDS) scores ranging 0-10. Sporadic CMT2A were selected on the basis of the disease severity and onset age. Clinical and molecular genetic analysis screening were done by applying multiplex polymerase chain reaction (PCR) to identify CMT1A type. Patients suffering from CMT2A were identified by Sanger sequencing method Based on frequency of mutation in various domains of MFN2 gene, three mutations were selected for structural analysis of the protein. These were: p. Arg94Trp, His165Arg and p. Thr362Met

For molecular analysis, capillary sequencing was performed for all the exons of the MFN2 gene. The screening was performed by sequencing the entire coding region. Samples were analysed by capillary sequencing. Sequences of MFN2 exons were determined by Sanger's sequencing method using automatic genetic analyser (ABI3130XL; Applied Biosystems, Foster City, CA). Determination of causative mutations and in silico analysis candidate variants considered causative were confirmed by Sanger's sequencing with extended members of respective families.[19]

In order to check any structure variations due to these mutations, the structure of wild type (WT) and mutated sequences were predicted. Due to unavailability of a suitable template for homology modelling, the structures were predicted using threading approach. To predict the structures, an alignment algorithm was used.[19] To check the variation in the structures due to the mutations, the structures of mutated proteins were superimposed against WT. The superimposition was performed using a unified platform for automated protein structure and function prediction.[20] The predicted and superimposed structures were visualised and coloured using Pymol version 2.1.1.

Result

Of the 10 patients, 5 (50%) were in each of the two groups.

Table: Quantification of disease severity. Patients with Mitofusin 2 (MFN2) mutations were divided into two categories by onset age (early onset< 10 years or late onset > 10 years). The early onset group was found to be associated with severe functional.

sr #	Gene	Phenotype	amino acid change	Domain	sex	onset Age	CMTNS	FDS
	Early onset(10 years)							
1			Arg94Trp			4	11	1
2		CMT2A	Arg94Trp	GTPase	Female	8	24	6
3		CMT2A	His165Arg	GTPase	Male	5	11	2
4		CMT2A	THr362Met	GTPase	Female	8	23	3
	Late onset > (10 years)				Female			
5		CMT2A	His165Arg	GTPase	Male	50	5	2
6		CMT2A	His165Arg		Female	10	18	3
7		CMT2A	His165Arg	GTPase	Female	14	5	1
8		CMT2A	His165Arg	GTPase	Female	15	X	3
9		CMT2A	Arg94Trp	GTPase	Female	31	9	2
10		CMT2A	Arg94Trp	GTPase	Female	15	8	2

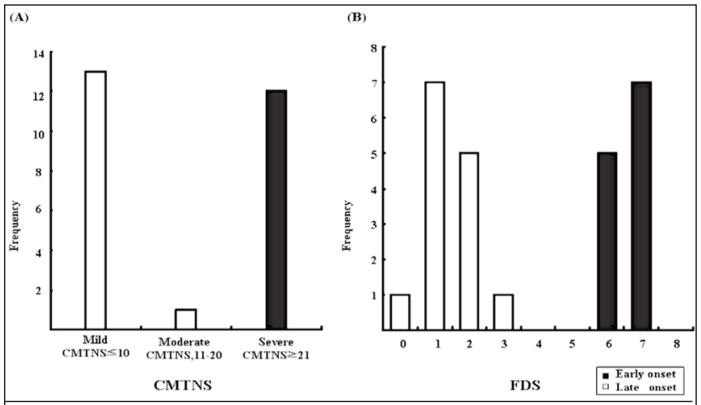

Figure-1: Quantification of disease severity. Patients with Mitofusin 2 (MFN2) mutations were divided into two categories by onset age (early onset< 10 years or late onset > 10 years). The early onset group was found to be associated with severe functional disability (Functional disability Scale [FDS] = 6-7) and the late-onset group with asymptomatic to mild disease forms (FDS <3).

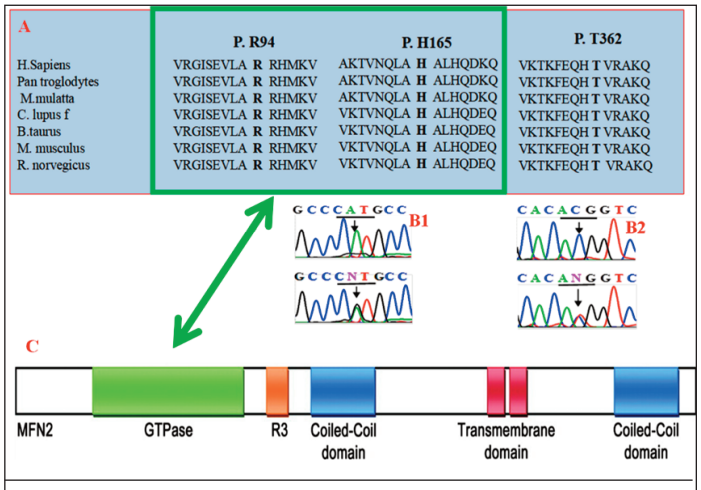

Figure-2: (A) Mitofusin 2 (MFN2) mutations and their conservation in species. (B) Conservation of amino acids at mutation sites in different species. Mutation sites are indicated by arrows.(C) Various domains of the MFN2 gene.

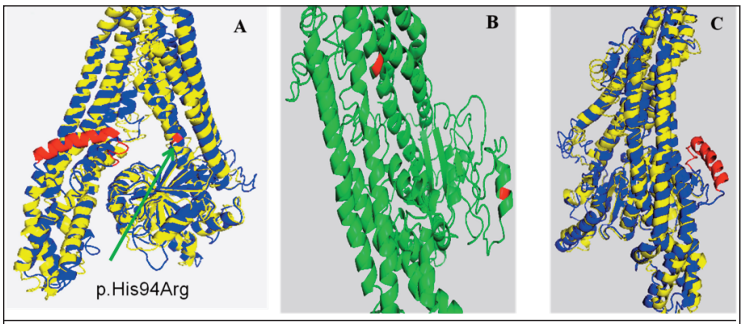

Figure-3: (A) Amino acid position p.R94 showed an extra conformational helix at alpha helix of Mitofusin 2 (MFN2) gene. The superimposed images of wild type (WT) and mutated protein. The change in the structure was predicted in the mutated protein at position 94. Due to the mutation, an extra alpha helix was formed in the mutated protein (highlighted red). Green = WT, Blue = Mutated protein. (B) Mutated amino acid at position p.165 and p.362 was without structural modification (C) The mutant and WT structure with conformationly modified alpha helix.

Overall, there were 7 (70%) females and 3(30%) males (Table). Based on FDS scores, group A was associated with severe functional disability (Figure 1). MFN2 mutations and their conservation in species as well as various domains of the MFN2 gene were worked out (Figure 2). Change in the structure was predicted in the mutated protein at position p. Arg94Trp, and, due to the mutation, an extra alpha helix was formed in the mutated protein (Figure 3).

Discussion

Neuromuscular disorders is a broad-range term which includes a group of various diseases. CMT disease is one of the most common heterogeneous inherited disorders with symptoms of distal muscle weakness, optic atrophy and some other deformities in distal organs.

MFN2 is an essential component for mitochondrial machinery particularly in fusion mechanism.[21] The present study was intended at investigating the effect of three pathogenic and most frequent mutations responsible for CMT2A disorder and to see it in the light of various population of the world.[22] Two of the mutations were in GTPase domain and the third was in the R3 domain of MFN2 gene.[23] GTPase is highly conserved and mainly involved in mitochondrial fusion in mammalian cells. As a result, mutations in this region are responsible for various disease phenotypes.[24] Mutations found in GTPase domain exhibits a wide range of disease severity even the same mutation in various patients' shows the different severity. Mitochondrial fusion is an orchastreal activity of outer and inner membrane. Three large GTPase domains of MF1, MFN2 and OPA1 are required for mitochondrial fusion. So we suggest that the mutation in any of the three GTPase domain may affect the normal function of the rest of the two domains MFN2 is an essential component for mitochondrial machinery particularly in fusion mechanism. The present work was intended at investigating the effect of three pathogenic and most frequent mutations responsible for CMT type disorder in various populations of the world.[22] Two of the mutations were in GTPase domain and third was in R3 domain of MFN2 gene.[23] GTPase is highly conserved and mainly involved in mitochondrial fusion in mammalian cells. As a result mutations in this regions are responsible various disease phenotypes. The current study found that one of the mutations present in GTPase domain p.94 was responsible for conformational changes in structure of the MFN2 gene which is line with literature.[25] We found that MFN2, but not MFN1 (Homo Sapiens), is required for proper development and maintenance of the cerebellum. Purkinje cells require MFN2 for their extensive dendritic outgrowth and survival. Genetic studies in flies suggest that neurons require abundant mitochondria at nerve termini to maintain synaptic transmission and proper ultrastructure.

Conclusion

Change in the structure of protein can be in a critical position that is involved in the mitochondrial fusion process. However, further studies are required to validate and explain the findings.

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Conflict of Interest: None.

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