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Functional Analysis of Rheumatoid Arthritis Associated Mutations on Pyrin b30.2 Domain

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Abstract—*Rheumatoid Arthritis (RA) is an auto immune, comorbid disorder that attacks the synovial joints in symmetric order and ultimately leads to the loss of function. Aetiology of the disease is not completely understood. Genetic studies indicated the significant role of HLA-DRB1 and others towards disease pathogenesis. Extensive studies have been carried out on RA associated mutations that revealed the potential role of MEFV gene. Pyrin (MEFV gene product) is thought to activate the maturation of pro-inflammatory cytokine such as IL1β. Pyrin mutations like M694V and V726A, reported during the familial Mediterranean fever (FMF), also plays key role in RA. Further analysis implicates that both the mutations are associated with pyrin b30.2 domain. But the effect of these mutations on domain stability and function is not yet studied. Hence, we hypothesize that the presence of these mutants could significantly determine the disease progression by affecting the protein structure and stability. Thus, the objective of the present study is to incorporate both the mutations computationally in b30.2 domain and to scrutinize their effects. Mutations were induced manually in the protein structure using Mutate module of Swiss PDB Viewer (SPDBV) and their subsequent effects were determined by SNAP, I-Mutant 2.0, PolyPhen2.0.9, MuPro, PrDOS and IUPred servers. The approach has enabled us to analyse the potential effects of mutations on protein and thus could provide a possible explanation for their occurrence and role in RA pathogenesis.*

1. INTRODUCTION

Rheumatoid arthritis is a chronic systemic autoimmune disease that causes symmetric polyarticular inflammation of synovium leading to permanent joint destruction and deformity [1]. Development of RA in one may be ascribed to an individual's genetic and environmental factors. Recent genetic studies have identified some genetic risk factors associated with RA such as HLA DRB 1 and PTPN 22 (Protein tyrosine phosphatise 22) gene [2, 3]. Moreover, mutations in genes could also significantly increase the likelihood for RA development. Interestingly, one such mutational study has discovered an association of Mediterranean fever (MEFV) gene with RA [4]. MEFV gene is located on chromosome 16 at position 13.3. The gene is known to express in peripheral blood leukocytes and human synovial fibroblasts where it encodes a protein called pyrin [5, 6]. Pyrin is a cytoplasmic protein with an N-terminal domain similar in secondary structure to the death domain of transducers of apoptotic and inflammatory signals.

Various reports suggest the active involvement of pyrin in innate immunity and inflammation. However, its contribution in inflammatory pathways is not yet determined as it has uncertain role in IL-1β activation [7-9]. Pyrin seems to regulate pro-interleukin (IL) 1β production by caspase 1, which gets activated upon binding with apoptotic speck protein (ASP) [8]. Presence of mutations like M694V and V726A in MEFV gene leads to a hereditary periodic fever syndrome, Familial Mediterranean Fever (FMF). Surprisingly, the association and overrepresentation of the M694V mutation in a cohort with juvenile RA has also been reported [10]. According to few reports, the over expressed IL1β causes impairment in the regulation of neutrophil and other leukocyte activation that eventually results in bursts of systemic inflammation [11].

Molecular analysis of the protein revealed that in contrast to its N-terminal domain, the ~ 200 amino acid residue long C-terminal domain (B30.2) is associated with higher mutation rates [8]. However, the exact role of Pyrin B30.2 domain is not understood, in others this conserved domain is believed to be involved in protein-protein interactions [12]. Keeping in mind, the involvement of pyrin in inflammation and occurrence of RA associated mutations in its B30.2 domain. Our study focuses on to analyse various structural and functional impacts caused by the presence of mutations (M694V and V726A) on protein B30.2 domain. Thus, the study could provide a better understanding for plausible complex mechanisms underlying the disease (RA) pathogenesis and progression.

2. MATERIAL AND METHODS

2.1 Preparing target molecule

To explore the mutational effect on the protein, we have downloaded the X- ray crystallographic structure of pyrin b30.2 domian (PDB ID: 2WL1) of pyrin protein from Protein Data Bank (http://www.rcsb.org/pdb).

2.2 Mutational screening and analysis

Total three mutations (M694V, E148Q, and V726A) of pyrin protein are reported in case of RA [13]. Out of these three,

only two mutations (M694V and V726A) were selected as they were the part of one of the most important functional domain (pyrin b30.2) of pyrin. Presence of both the mutations on this domain can have great impact on both the structure and functionality of the protein and thus the pathways involved in. Mutational analysis was carried out by inducing the mutation to the protein domain. Mutate tool of Swiss PDB Viewer (SPDBV) was used to induce single amino acid substitution to PDB structure of pyrinb30.2 domain.

The effect of point mutation on protein functionality and stability was analysed using different servers such as SNAP, PrDos, IUPred, MuPro, I-Mutant 2.0 and PloyPhen.

2.3 Effect on protein stability

SNAP server (http://www.rostlab.org/services/SNAP) predicts the effects of point mutation on protein functionality. It gives output in the form reliability index and expected accuracy of the prediction [14]. Reliability index should be ≥ 0 and expected accuracy should be $\geq 50\%$.

2.4 Effect on protein function

MuPro server (http://www.igb.uci.edu/servers/servers.html) was used to analyse the effect of substitution on protein stability. This server used support vector machine to evaluate protein stability with 80% accuracy [15]. I-Mutant 2.0 server (http://gpcr.biocomp.unibo.it/cgi/predictors/I-Mutant2.0/I Mutant2.0.cgi) predicts the DeltaDeltaG (DDG) value before and after mutation using support vector machine [16]. This value estimated through equation: DGf wt - DGf mut. Where, wt stands for wild type and mut is mutated protein.

2.5 Disordered region analysis

Analysing the effect of mutations and structural disorder on pyrin protein has been carried out by IUPred and PrDOS servers. IUPred server (http://iupred.enzim.hu) identifies the unstructured region of protein based on estimated energy content [17]. PrDOS server (http://prdos.hgc.jp) predicts the disordered region of protein from the sequence and displays its disorder probability [18].

3. RESULTS AND DISCUSSION

Pyrin regulates IL-1β production by directly interacting with caspase 1 through its N-terminal PYRIN motif. But, this interaction is directly carried out through C terminal pyrin b30.2 domain of pyrin. Mutations in this domain reduce the binding and interaction with caspase 1[8]. IL-1 is very important player of pathogenesis of RA whose high levels are found in case of arthritis [19].

3.1 Impact of mutations on function and stability

Structure of protein was downloaded from RCSB (Fig. 1) and mutated with the help of Mutate tool of SPDBV. SNAP results indicated that M694V can affect the function of the protein. The prediction for this mutation was non-neutral showing the reliability index 3 and expected accuracy of 78%. However, V726A was identified as the neutral mutation with reliability index 4 and expected accuracy 85%. Predicting the effect of both the mutations on protein stability was carried out by MuPro and I- Mutant2.0 servers. Results of MuPro server revealed the decrease in the stability of protein structure in Support Vector Machine (SVM) based methods with confidence score -1 for both the mutations. Similarly, Neural network based method indicated the decrease in protein stability with confidence scores -0.99327 and -0.99492 for M694V and V726A respectively. I- Mutant2.0 server was also utilized for calculating the change in protein stability. The output provided the DDG score of -0.38 for M694V and -1.61 for V726A. Score towards negative side (below 0) shows decrease in stability whereas; score towards positive side (above 0) shows an increase in stability. Thus, M694V and V726A mutation may have effect on the stability of the structure as indicated by its negative score.

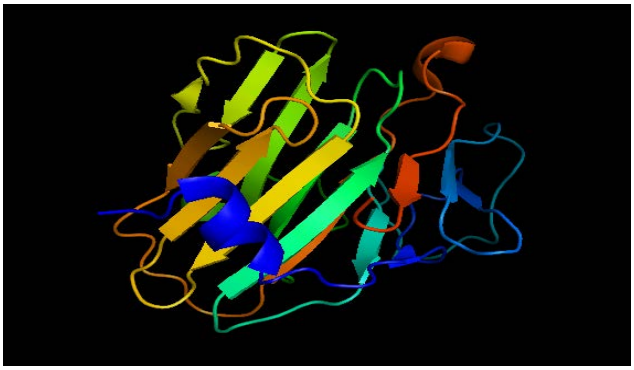

Fig. 1: PyMol view of pyrin30.2 domain (Blue colour to red colour is representing the N terminal to C- terminal end respectively).

3.2 Protein disorder analysis

Mutations majorly cause the loss of secondary structure of a protein. So, predictions made by IUPred server indicated that mutations M694V and V726A have disorder tendency of 0.2129 and 0.0592 respectively. Disordered tendency of mutating residues was calculated on the scale of 0-1.00. In the present study mutating residues showed low disorder tendency. PrDOS server showed residues 1-3 are in disordered regions. Mutating residues were not found in the disordered region of the protein (Fig. PrDos).

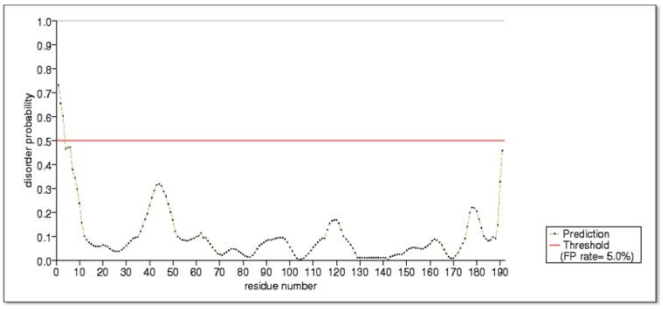

Fig. 2: Plot representing the disordered region of protein.

4. CONCLUSION

Our results have shown that single amino acid substitutions such as M694V and V726A have strong structural and functional effects on pyrinB30.2 domain of pyrin protein. Pyrin helps in IL-1 beta production on interaction with caspase 1 through its B30.2 domain. These mutations will remarkably decrease the stability of protein and thus its binding ability with caspase 1 will decrease. Decreased binding with caspase-1 will interfere with IL-1 β production which have crucial role in RA pathogenesis. Thus, our study suggests that the presence of these mutations can affect disease progression.

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