Computational analysis of adverse missense mutations of HLA-B27 protein

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ABSTRACT

The detrimental missense mutations of HLA-B27 gene causing Ankylosing spondylitis were identified computationally and the substrate binding efficiencies of these mutations were analyzed. Out of 12 variants, I-Mutant 3.0, SIFT and PolyPhen programs identified 1 variant (Y83H) that was less stable, deleterious as well as damaging respectively. Modeling of this one variant was performed to understand the changes in their conformations with respect to the native HLA-B27 protein by computing their RMSD and Total energy. Furthermore the native and the variant were docked with beta-microglobulin to explain the binding efficiencies of those detrimental missense mutations.

Key words: HLA-B27 gene, missense mutation, Ankylosing spondylitis

INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammation of the sacroiliac joints, spine and peripheral joints. The development of ankylosing spondylitis is still unclear. Genetics factors such as human leukocyte antigen HLA-B27 and ERAP1 have been widely reported to associate to AS susceptibility [1]. HLA-B27 is present in 90-95 % of patients with ankylosing spondylitis [2]. Human Leukocyte Antigen (HLA) B27 is a class I surface antigen encoded by the B locus in the major histocompatibility complex (MHC) on chromosome 6 and presents antigenic peptides (derived from self and non-self antigens) to T cells. HLA-B27 is strongly associated with ankylosing spondylitis (AS), and other associated inflammatory diseases referred to as spondyloarthopathies. Psoriatic arthritis is classified within the spondyloarthritides. It is defined as a chronic inflammatory disease of synovial joints associated with psoriasis and usually rheumatoid factor negative [3]. In 1977 a group of psoriatic patients positive for HLA-B27 who were at increased risk of developing axial and peripheral arthritis including distal interphalangeal involvement was described [2].

Each individual possesses unique characteristics reflecting their genotype. For example, almost all nucleotide bases (99.9%) are exactly the same in all people; however, the remaining 0.1% account for ~1.4 million individual-specific differences (single nucleotide polymorphism, SNP) that occur in humans. Single nucleotide polymorphisms are a common type of genetic variation, describing changes in a single DNA building block, called nucleotide. These differences may be within the coding or non-coding regions of DNA and may or may not result in amino acid changes, which, in turn, can either be harmless or disease causing [4]. The effects caused by nsSNPs can be broadly grouped into four distinctive categories [5] (although the effects may be mutually dependent) depending on what
type of system or process have been affected by nsSNPs: 1) protein folding, stability, flexibility, and aggregation; 2) functional sites, reaction kinetics, and dependence on the environmental parameters, such as pH, salt concentration, and temperature; 3) protein expression and subcellular localization; and 4) protein-small molecule, protein-protein, protein-DNA, and protein-membrane interactions [6].

However, the goals of our study are to computationally investigate: 1) the possibility that disease-causing and harmless nsSNPs affect protein-protein interactions differently and 2) to reveal the basic principles of the effects of naturally occurring interfacial nsSNPs on protein-protein interactions. The rationale behind our approach is that any miss-sense mutation of amino acid could somehow affect the binding energy, stability and energy of the protein and even harmless nsSNPs can also cause dramatic changes in the phenotype resulting in natural differences among individuals.

MATERIALS AND METHODS

2.1 Datasets
The protein sequence and variants (single amino acid polymorphisms/missense mutations/point mutations) of HLA-B27 were obtained from the Swissprot database available at http://www.expasy.ch/sprot/. The subsection of each Swissprot entry provided information on polymorphic variants, some of which polymorphic variants might be disease(s) - associated by causing defects in a given protein; most of them were nsSNPs (non-synonymous SNPs) in the gene sequence and SAPs (single amino acid polymorphisms) in the protein sequence [7][8][9]. The 3D Cartesian coordinates of HLA-B27 protein and its complex were obtained from Protein Data Bank with PDB IDs 2BST [10] for in silico mutation modeling and docking studies based on detrimental point mutants.

2.2 Predicting Stability Changes caused by SAPs using Support Vector Machine (IMutant3.0)
I-Mutant, a suite of Support Vector Machine based predictors integrated in an unique web server. It offered the opportunity to predict automatically protein stability changes upon single- site mutations starting from protein sequence alone or protein structure when available. Moreover it provided the possibility to predict human deleterious Single Nucleotide Polymorphism starting from the protein sequence alone. Users could choose among three different predictors:

- The first was a SVM based predictor for protein stability changes upon single point protein mutation starting from structural informations.
- The second one was a SVM based predictors for protein stability changes upon single point protein mutation starting from sequence informations I-Mutant -ΔΔG.
- The third one was a SVM based predictor for human Deleterious Single Nucleotide Polymorphism starting from sequence informations I-Mutant-Disease.

2.3 Analysis of Functional Consequences of Point Mutations by a Sequence Homology-Based Method (SIFT)
We used the program SIFT [11] available at http://blocks.fhcrc.org/sift/SIFT.html, which specifically detected deleterious single amino acid polymorphisms. SIFT, a sequence homology-based tool presumed that important amino acids would be conserved in a protein family; therefore, changes at well-conserved positions tend to be predicted as deleterious [12]. Queries were submitted in the form of protein sequences. SIFT took a query sequence and used multiple alignment information to predict tolerated and deleterious substitutions for every position of the query sequence. SIFT being a multistep procedure that, for given a protein sequence, (a) searched for similar sequences, (b) chooses closely related sequences that may share similar function, (c) obtained the multiple alignments of these chosen sequences, and (d) calculated normalized probabilities for all possible substitutions at each position from the alignment. Substitutions at each position with normalized probabilities less than a chosen cutoff were predicted to be deleterious and those greater than or equal to the cutoff were predicted to be tolerated. The cutoff value in SIFT program was a tolerance index of ≥ 0.05. The higher the tolerance index, the less functional impact a particular amino acid substitution would be likely to have.

2.4 Simulation for Functional Change in a Point Mutant by Structure Homology- Based Method (PolyPhen)
Analyzing the damage caused by point mutations at the structural level was considered very important to understand the functional activity of the protein. We used the server PolyPhen [13] available at http://coot.embl.de/PolyPhen/. Input options for the PolyPhen server were protein sequence, SWALL database ID or accession number, together with the sequence position of two amino acid variants. The query was submitted in the form of a protein sequence
with a mutational position and two amino acid variants. Sequence-based characterization of the substitution site, profile analysis of homologous sequences, and mapping of the substitution site to known protein 3D structures were the parameters taken into account by PolyPhen server to calculate the score. It calculated position-specific independent counts (PSIC) scores for each of the two variants and then computed the PSIC score difference between them. The higher the PSIC score difference, the higher the functional impact a particular amino acid substitution would be likely to have.

2.5 Modeling Single Amino Acid Polymorphism Location on Protein Structure to Compute Total Energy and RMSD
Structural analysis was performed for computing the total energy and evaluating the structural deviation between native type and mutant types by means of RMSD (Root Mean square Deviation). We used the web resource Protein Data Bank and Single Amino Acid Polymorphism database (SAAPdb) [14] to identify the 3D structure of HLA-B27 protein. We also confirmed the mutation position and the mutated residue in PDB ID 2BST. The mutation was performed by using SWISSPDB viewer and the energy minimization for 3D structures was performed by NOMAD-Ref server [15]. This server use Gromacs as default force field for energy minimization based on the methods of steepest descent, conjugate gradient and L-BFGS methods [16]. We used the conjugate gradient method to minimize the energy of the 3D structure of HLA-B27 protein. To optimize the 3D structure of HLA-B27 protein, we used the ifold server [17] for simulated annealing, based on discrete molecular dynamics and being one of the fastest strategies for simulating protein dynamics. This server efficiently sampled the vast conformational space of biomolecules in both length and time scales. Divergence of the mutant structure from the native structure could be caused by substitutions, deletions and insertions [18] and the deviation between the two structures could alter the functional activity [19] with respect to the binding efficiency of the inhibitors, which was evaluated by their RMSD values.

2.6 Identifying stabilizing residues in proteins (SRide)
Residues expected to play key roles in the stabilization of proteins [stabilizing residues (SRs)] were selected by combining several methods based mainly on the interactions of a given residue with its spatial, rather than its sequential neighbourhood and by considering the evolutionary conservation of the residues. A residue was selected as a stabilizing residue if it had high surrounding hydrophobicity, high long-range order and high conservation score and also if it belongs to a stabilization center. The definition of all these parameters and the thresholds used to identify the SRs were discussed in detail. SRs could be applied in protein engineering and homology modeling and could also help to explain certain folds with significant stability.

2.7 Analysis of secondary structure elements of native and mutant
We used STRIDE web server for analysis of secondary structure of native and mutant. STRIDE, an automatic algorithm for protein secondary structure assignment from atomic coordinates implemented a knowledge-based algorithm that made combined use of hydrogen bond energy and statistically derived backbone torsional angle information and was optimized to return resulting assignments in maximal agreement with crystallographers’ designations. The STRIDE web server provided access to this tool and allowed visualization of the secondary structure, as well as contact and Ramachandran maps for any file uploaded by the user with atomic coordinates in the Protein Data Bank (PDB) format [20]. STRIDE considered both hydrogen bonding patterns and backbone geometry. The hydrogen bond energy was calculated using an empirical energy function which took into account the distance between the donor and the acceptor and the deviations from linearity of the bond angles. A weighted product of hydrogen bond energy and torsion angle probabilities for a-helix and b-sheet was used to determine the start and stop positions of secondary structure elements based on empirically optimized recognition thresholds.

2.8 Identification of Binding Sites and Computation of Atomic Contact Energy (ACE) between HLA-B27 protein and its substrate
To compute the ACE between HLA-B27 protein and its substrate, we used the program PatchDock for docking the native and mutant HLA-B27 protein with beta-microglobulin to compute the ACE by using additional option of binding residue parameter. The underlying principle of this server was based on molecular shape representation, surface patch matching, filtering and scoring [21]. It found docking transformations that yield good molecular shape complementarities. Such transformations, when applied, induced both wide interface areas and small amounts of steric clashes. A wide interface ensured with the aim of including several matched local features of the docked molecules, which had complementary characteristics. The PatchDock algorithm divided the Connolly dot surface representation [22] of the molecules into concave, convex and flat patches. Then, complementary patches were
matched to generate candidate transformations. Each candidate transformation was further evaluated by a scoring function that considered both geometric fit and atomic desolvation energy [23] [24]. Finally, an RMSD clustering was applied to the candidate solutions to discard redundant solutions. The main reason behind Patch Dock’s high efficiency was its fast transformational search, which was driven by local feature matching rather than by brute force searching of the six dimensional transformation spaces. It further speeded up the computational processing time using advanced data structures and spatial pattern detection techniques, such as geometric hashing and poses clustering.

RESULTS AND DISCUSSION

3.1 The SAP Data Set from Swissprot
The HLA-B27 protein and 12 variants from A-chain of the protein, namely, A65T, Y83H, D101N, D101S, T104N, N121S, H138D, D140H, D140Y, S155R, V176E and A235G investigated in this work were retrieved from the Swissprot database.

3.2 Deleterious Single Point Mutants Identified by the SIFT Program
The degree of conservation of a particular position in a protein was determined using sequence homology based tool SIFT. The protein sequences of the 12 variants were submitted to SIFT to determine their tolerance indices. As the tolerance level increases, the functional influence of the amino acid substitution decreases and vice versa. Among the 12 variants, 2 variants were found to be deleterious, having tolerance index scores of ≤0.05 (Table 1). Both the variants showed a very high deleterious tolerance index score of 0.00.

3.3 Damaging Single Point Mutations identified by the PolyPhen Server
Structural level alterations were determined by PolyPhen program. Protein sequence with mutational position and amino acid variants associated with the 12 single point mutants were submitted to the PolyPhen server. A PSIC score difference of 0.5 and above was considered to be damaging. It could be seen from Table 1 that, out of 12 variants, 1 was considered to be damaging by PolyPhen. This variant exhibited a PSIC score difference of 0.998. This variant was also found to have high tolerance index score by SIFT program.

3.4 Identification of Functional Variants by I-mutant 3.0
Of the 12 variants, 10 variants were found to be less stable using the I-Mutant 3.0 server (Table 1). Among these 10 variants, one variant showed a ΔΔG value > -1.0 and nine variants showed a ΔΔG value < -1.0 as depicted in (Table 1). Of the ten variants that showed a negative ΔΔG, two variants (N121S and A235G) retained their amino acid properties. Two variants (Y83H and S155R) changed from polar uncharged to polar positively charged and one (T104N) from polar uncharged to polar negatively charged. Three variants (D101N, D101S and D140Y) changed from negatively charged to uncharged. One variant (A65T) changed from non-polar to polar uncharged and one (V176E) from non-polar to polar negatively charged. Variant H138D changed from positively charged to negatively charged and D140H from negatively charged to positively charged. Indeed, by considering only amino acid substitution based on physico-chemical properties, we could not be able to identify the detrimental effect. Rather, by considering the sequence conservation along with the above said properties could have more advantages and reliable to find out the detrimental effect of missense mutations.

Table-1 List of functionally significant mutants predicted by SIFT, PolyPhen and I-Mutant 3.0

<table>
<thead>
<tr>
<th>Variants</th>
<th>Tolerance index</th>
<th>PSIC SD</th>
<th>ΔΔG</th>
</tr>
</thead>
<tbody>
<tr>
<td>A65T</td>
<td>0.18</td>
<td>0.000</td>
<td>-0.84</td>
</tr>
<tr>
<td>Y83H</td>
<td>0.00</td>
<td>0.998</td>
<td>-0.77</td>
</tr>
<tr>
<td>D101N</td>
<td>1.00</td>
<td>0.000</td>
<td>-0.53</td>
</tr>
<tr>
<td>D101S</td>
<td>0.53</td>
<td>0.000</td>
<td>-0.52</td>
</tr>
<tr>
<td>T104N</td>
<td>0.39</td>
<td>0.000</td>
<td>-0.94</td>
</tr>
<tr>
<td>N121S</td>
<td>0.84</td>
<td>0.000</td>
<td>-0.29</td>
</tr>
<tr>
<td>H138D</td>
<td>0.36</td>
<td>0.000</td>
<td>-0.64</td>
</tr>
<tr>
<td>D140H</td>
<td>0.48</td>
<td>0.002</td>
<td>-0.31</td>
</tr>
<tr>
<td>D140Y</td>
<td>1.00</td>
<td>0.007</td>
<td>+0.11</td>
</tr>
<tr>
<td>S155R</td>
<td>1.00</td>
<td>0.001</td>
<td>+0.18</td>
</tr>
<tr>
<td>V176E</td>
<td>0.50</td>
<td>0.000</td>
<td>-0.52</td>
</tr>
<tr>
<td>A235G</td>
<td>0.00</td>
<td>0.163</td>
<td>-1.27</td>
</tr>
</tbody>
</table>
3.5 Rational Consideration of Detrimental Point Mutations

We rationally considered one most potential detrimental point mutation (Y83H) for further course of investigations because it was commonly found to be less stable, deleterious, and damaging by the I-Mutant3.0, SIFT and PolyPhen servers respectively. We considered the statistical accuracy of these three programs, I-Mutant improved the quality of the prediction of the free energy change caused by single point protein mutations by adopting a hypothesis of thermodynamic reversibility of the existing experimental data. The accuracy of prediction for sequence and structure based values were 78% and 84% with correlation coefficient of 0.56 and 0.69, respectively [25]. SIFT correctly predicted 69% of the substitutions associated with the disease that affect protein function. PolyPhen-2 evaluates rare alleles at loci potentially involved in complex phenotypes, densely mapped regions identified by genome-wide association studies, and analyses natural selection from sequence data, where even mildly deleterious alleles must be treated as damaging. PolyPhen-2 was reported to achieve a rate of true positive predictions of 92% [25][26][27]. To obtain precise and accurate measures of the detrimental effect of our variants, comprehensive parameters of all these three programs could be more significant than individual tool parameters. Hence, we further investigated this detrimental missense mutation by structural analysis.

3.6 Computing Total Energy and RMSD by Modelling of Mutant Structures

Mapping the one variant namely, Y83H into HLA-B27 protein structure information was obtained from SAAPdb. The available structure for HLA-B27 protein got a PDB ID 2BST. The mutational position and amino acid variants were mapped in the native structure. Mutation at specified position was performed by SWISSPDB viewer independently to get modeled structures. Then, energy minimization was performed by the NOMAD - Ref server for both the native structure (PDB 2BST) and mutant modeled structures. In order to find out the structural stability of HLA-B27 protein of native and mutant, we computed the total energy, which included bonds, angles, and torsions, non-bonded and electro-static constraints from GROMOS 96 force field implemented in DeepView to check their stability. It could be seen from Table 2 that the total energy of the native protein had -19285.05KJ/Mol whereas all the mutants had the total energy lower than native protein. The lower the total energy, greater was the stability of the protein structure. In order to find out the deviation between the two structures, we superimposed the native structure (PDB 2BST) with all the mutant modeled structures to get the RMSD. The higher was the RMSD value, more the deviation between the native and mutant structure, which in turn change the binding efficiency with its interacting partners due to deviation in the 3D space of the binding residues of HLA-B27 protein. Table 2 showed the RMSD for native structure with all the mutant modeled structures. Figure 2 showed the superimposed structure of native HLA-B27 protein (green) with mutant Y83H (Blue).
Figure 2. Structure of native HLA-B27 protein (green), mutant Y83H (Blue) and superimposed structure of native HLA-B27 with mutant Y83H structure showing RMSD of 5.97 Å.

Table 2 Total energy, RMSD (Å) and Stabilizing Residues

<table>
<thead>
<tr>
<th>Variants</th>
<th>ENERGY (KJ/mol)</th>
<th>RMSD (Å)</th>
<th>Stabilizing Residues</th>
<th>No. of Stabilizing Residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>-19285.0</td>
<td>0</td>
<td>GLY112, LEU168, GLY207, PHE208, LEU215, GLN242, ALA245, CYS259</td>
<td>8</td>
</tr>
<tr>
<td>A65T</td>
<td>-19484.6</td>
<td>2.16</td>
<td>SER38, GLY112, LEU168, GLY207, PHE208, LEU215, GLN242, ALA245, CYS259</td>
<td>9</td>
</tr>
<tr>
<td>Y83H</td>
<td>-18413.1</td>
<td>3.97</td>
<td>PHE33, CYS101, GLY112, ALA205, ALA245, CYS259</td>
<td>6</td>
</tr>
<tr>
<td>D101N</td>
<td>-19640.7</td>
<td>2.11</td>
<td>GLY112, PHE208, LEU215, GLN242, ALA245, CYS259</td>
<td>6</td>
</tr>
<tr>
<td>D101S</td>
<td>-19118.9</td>
<td>2.60</td>
<td>SER38, GLY112, LEU168, GLY207, PHE208, LEU215, GLN242, ALA245, CYS259</td>
<td>9</td>
</tr>
<tr>
<td>T104N</td>
<td>-19647.2</td>
<td>2.65</td>
<td>VAL28, SER38, LEU168, PHE208, LEU215, GLN242, ALA245, CYS259</td>
<td>8</td>
</tr>
<tr>
<td>N121S</td>
<td>-19713.6</td>
<td>2.61</td>
<td>ARG6, ASP30, SER38, GLY112, PHE208, LEU215, GLN242, LYS243, ALA245, CYS259</td>
<td>10</td>
</tr>
<tr>
<td>H138D</td>
<td>-19888.5</td>
<td>2.97</td>
<td>ASP30, PHE208, LEU215, LYS243, ALA245, CYS259</td>
<td>6</td>
</tr>
<tr>
<td>D140H</td>
<td>-19592.6</td>
<td>2.52</td>
<td>ASP30, ALA205, PHE208, LEU215, GLN242, ALA245, CYS259</td>
<td>7</td>
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<tr>
<td>D140Y</td>
<td>-19710.8</td>
<td>2.94</td>
<td>ASP30, CYS101, GLY112, PHE208, LEU215, GLN242, LYS243, ALA245, CYS259</td>
<td>9</td>
</tr>
<tr>
<td>S155R</td>
<td>-20074.9</td>
<td>2.95</td>
<td>ARG6, VAL28, ASP29, CYS101, GLY112, GLY207, PHE208, LEU215, ALA245, CYS259</td>
<td>10</td>
</tr>
<tr>
<td>V176E</td>
<td>-19521.5</td>
<td>2.51</td>
<td>SER38, CYS101, GLY207, PHE208, LEU215, GLN242, ALA245, CYS259</td>
<td>8</td>
</tr>
<tr>
<td>A235G</td>
<td>-20031.2</td>
<td>2.78</td>
<td>ASP30, SER38, GLY112, GLY207, PHE208, LEU215, LYS243, ALA245, CYS259</td>
<td>9</td>
</tr>
</tbody>
</table>

3.7 Computing the Stabilizing residues in HLA-B27 protein

We further evaluated the stability of protein structure by using the SRide server to identify the number of stabilizing residues for both native and mutant structures. The parameters used for identifying the Stabilizing residues were surrounding hydrophobicity, long-range order, stabilization center and conservation score. Based on this analysis,
we found that a total number of stabilizing residues in the native structure of HLA-B27 protein were 8. On the other hand, the mutant structure (Y83H) of HLA-B27 protein had 6 stabilizing residues as shown in Table 2.

This clearly indicated that the mutant changed its structure with increased energy and decreased stabilizing residues as compared to native. We further evaluated the effect of this detrimental missense mutation by studying the secondary structure elements of both native and mutant protein in order to understand the alteration of conformation of mutant structure as compared to native.

3.8 Analysis of secondary structure elements of native and mutant using STRIDE web server
We further evaluated the distribution of secondary structure elements in native and mutant protein (Y83H). Using the STRIDE web server we calculated the number of secondary structure elements for native and mutant structures. From table 3 we could see that the distribution of secondary structure elements in native was distributed as 27 coils, 49 turns, 116 strands, 71 alpha helixes, 11 \(3_{10}\)-helixes and 2 bridges whereas for variant (Y83H) the secondary structure elements were distributed as 26 coils, 52 turns, 116 strands, 69 alpha helixes, 11 \(3_{10}\)-helixes and 2 bridges. Since the distribution of secondary structure elements were altered in native and mutant specifically in coil, turn and alpha helix, this could be the reason for alteration of conformation of mutant structure (Table 3). We further analyzed the effect of this detrimental missense by performing binding analysis between HLA-B27 protein and beta-microglobulin through docking studies in order to understand the functional activity of HLA-B27 protein.

3.9 Investigating the Rationale of Binding Efficiency for Native and Mutant HLA-B27 protein with beta-microglobulin
In order to find out the binding efficiency of native and mutant HLA-B27 protein with its interacting partner beta-microglobulin, we implemented molecular modelling approach for rationalizing the functional activity of this mutant Y83H. In this analysis, we modeled a missense mutation (Y83H) in the chain A of the PDB ID 2BST by swisspdb viewer and energy minimization was performed for the entire complex (both native and mutant complex) by GROMACS (Nomad-ref) followed by simulated annealing to get the optimized structures using a discrete molecular dynamics approach (ifold).

Docking was performed using the PatchDock server between HLA-B27 protein and beta-microglobulin with both native and mutant modeled structures of HLA-B27 protein to find out the binding efficiency in the form of Atomic Contact Energy (ACE). By this analysis, we found that, the ACE between beta-microglobulin and native HLA-B27 protein was found to be \(-1.38\) kcal/mol, whereas with mutant, the ACE was found to be \(-0.52\) kcal/mol (Figure 3). Also, Figure 3 showed the docked complex of native and mutant (Y83H) HLA-B27 protein with beta-microglobulin. This data clearly portrayed that the maximum binding effect of beta-microglobulin with the native HLA-B27 protein might be due to the 3D conformation of beta-microglobulin which exclusively made a comfortable fit with less ACE into the 3D space of the binding residues of these native as compared to the mutant.
CONCLUSION

In this study, the HLA-B27 protein which has been found to be associated with Ankylosing spondylitis was investigated by computational analysis for deleterious missense mutations. Out of the 12 mutants in the alpha chain of HLA-B27 protein one was found to be damaging by PolyPhen server, two were predicted to be deleterious by SIFT and eight were found to be more stable than native protein by I-mutant3.0. Out of these one variant Y83H was common in all three programs. Thus, conversion of tyrosine to histidine at the 83rd position was found to be the major mutation in alpha chain of HLA-B27 protein which has also been showed in earlier wet lab studies by other group [28][29]. This variant was found to be less stable than the native protein which could be due to changes in secondary structure pattern of the protein as indicated by STRIDE results. The changes in secondary structure pattern may lead to misfolding of protein and loss of protein function. Docking analysis between beta-microglobulin and the native and mutant modeled structures generated Atomic Contact Energy scores -1.38 and -0.52 respectively which confirmed that the mutation was deleterious. We thus concluded that mutation of tyrosine to histidine at position 83 of alpha chain of HLA-B27 could be one of the causes of Ankylosing spondylitis associated with HLA-B27 protein.

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