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Identification and structural comparison of deleterious mutations in nsSNPs of ABL1 gene in chronic myeloid leukemia: A bio-informatics study

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Abstract

Single nucleotide polymorphism (SNP) serve as frequent genetic markers along the chromosome. They can, however, have important consequences for individual susceptibility to disease and reactions to medical treatment. Also, genetics of the human phenotype variation could be understood by knowing the functions of these SNPs. Currently, a vast literature exists reporting possible associations between SNPs and diseases. It is still a major challenge to identify the functional SNPs in a disease related gene. In this work, we have analyzed the genetic variation that can alter the expression and the function in chronic myeloid leukemia (CML) by ABL1 gene through computational methods. Out of the total 827 SNPs, 18 were found to be non-synonymous (nsSNPs). Among the 30 SNPs in the untranslated region, 3 SNPs were found in 5′ and 27 SNPs were found in 3′ untranslated regions (UTR). It was found that 16.7% nsSNPs were found to be damaging by both SIFT and PolyPhen server. UTR resource tool suggested that 6 out of 27 SNPs in the 3′ UTR region were functionally significant. The two major mutations that occurred in the native protein (1OPL) coded by ABL1 gene were at positions 159 (L → P) and 178 (G → S). Val (6), Ala (7) and Trp (344) were found to be stabilizing residues in the native protein (1OPL) coded by ABL1 gene. Even though all the three residues were found in the mutant protein 178 (G → S), only two of them Val (6) and Ala (7) were acting as stabilizing residue in another mutant 159 (L → P). We propose from the overall results obtained in this work that, both the mutations 159 (L → P) and 178 (G → S) should be considered important in the chronic myeloid leukemia caused by ABL1 gene. Our results on this computational study will find good application with the cancer biologist working on experimental protocols.

Keywords: Chronic myeloid leukemia (CML); ABL1 gene; nsSNP; 3′ UTR region; Deleterious mutations; SIFT; PolyPhen; Modeled structure; Stabilizing residue

1. Introduction

Genetic variation in the human genome is an emerging resource for studying cancer. We are in the initial stages of characterizing the tools (i.e., the single nucleotide polymorphism, SNP) to rigorously analyze the genetic contributions to complex diseases, such as cancer [1]. Single nucleotide polymorphisms, usually referred to as SNPs, are small genetic changes, single base nucleotides in DNA (individual As, Ts, Gs, or Cs), that vary among individuals. Human populations are estimated to be 99% identical at the level of genetic sequence. Diversity arises from the remaining 1% variation, most of which is accounted for by SNPs (a small percentage is due to deletions or insertions of DNA). There are estimated to be approximately 10 million SNPs in the human genome. They are found, on average, every 100–300 base pairs in the 3-billion-base pair genome, although their density varies between regions. SNPs are found in both coding and non-coding regions [2]. Among these, the non-synonymous SNPs (nsSNPs) cause changes in the amino acid residues. These are likely to be an important

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factor contributing to the functional diversity of the encoded proteins in the human population [3]. Out of many factors, the nsSNPs affect the gene regulation by altering DNA and transcriptional binding factor and in maintaining the structural integrity of cells and tissues [4]. Also nsSNPs affect the functional role of proteins in signal transduction of visual, hormonal and other stimulants [5,6].

Chronic myeloid leukemia (CML) is a pluripotent stem cell disease characterized by the presence of the Philadelphia (Ph′) chromosome in the leukemia cells of 96% of all CML patients [7]. The Philadelphia chromosome (Phi), hallmark of chronic myelogenous leukemia, is present in 5–20% of acute lymphoblastic leukemia's (ALL) and in rare cases (1–2%) of acute non-lymphoblastic leukemia's (ANLL) [8]. The t(9; 22) translocation results in the head-to-tail fusion of the BCR and ABL1 genes present in many cases of chronic myelogeneous leukemia. The human ABL1 oncogene has been mapped to a region of 225 kb on the long (q) arm of chromosome 9 [9]. The processes like cell differentiation, cell division, cell adhesion, and stress response are mediated by the ABL1 proto-oncogene which encodes a cytoplasmic and nuclear protein tyrosine kinase. The activity of c-Abl protein is negatively regulated by its SH3 domain, and deletion of the SH3 domain turns ABL1 into an oncogene [10]. The cell cycle function and DNA binding activity of ABL1 gene mainly depends on the regulation of CDC2-mediated phosphorylation by the expression of ABL1 tyrosine kinase. The ABL1 gene is expressed as either a 6- or 7-kb mRNA transcript, with alternatively spliced first exons, spliced to the common exons 2–11. Though our literature survey showed that, there is a wide choice of literature on ABL1 gene related to CML by experimental studies, computational analysis undertaken for an *in silico* investigations on the mutation of nsSNPs in ABL1 gene are scarce. We undertook this work mainly to perform computational analysis of the nsSNPs in ABL1 gene and to identify the possible mutations and proposed modeled structures for the mutant proteins and compared them with the native protein. We report that, the mutations at positions, namely, 159 (L → P) and 178 (G → S) in the native protein of ABL1 gene could be a candidate of major concern in the disease of chronic myeloid leukemia.

2. Materials and methods

2.1. Datasets

The SNPs and their related protein sequence for ABL1 gene were obtained from the dbSNP [11] http://www.ncbi.nlm.nih.gov/SNP/ for our computational analysis.

2.2. Analysis of functional consequences of coding nsSNPs by sequence homology based method (SIFT)

We used the program SIFT [12] available at http://blocks.fhcrc.org/sift/SIFT.html to detect the deleterious coding non-synonymous SNPs. SIFT is a sequence based homology tool which presumes that important amino acids will be conserved in the protein family, and so, changes at well-conserved positions tend to be predicted as deleterious. We submitted the query in the form of either SNPids or as protein sequences. The underlying principle of this program is that, SIFT takes a query sequence and uses multiple alignment information to predict tolerated and deleterious substitutions for every position of the query sequence. SIFT is a multistep procedure in the sense that, given a protein sequence, (1) searches for similar sequences, (2) chooses closely related sequences that may share similar function, (3) obtains the multiple alignment of these chosen sequences, and (4) calculates normalized probabilities for all possible substitutions at each position from the alignment. Substitutions at each position with normalized probabilities less than a chosen cutoff are predicted to be deleterious and those greater than or equal to the cutoff are predicted to be tolerated [13]. The cutoff value in SIFT program is tolerance index of $\geqslant 0.05$. Higher the tolerance index, less functional impact a particular amino acid substitution is likely to have.

2.3. Simulation for functional change in coding nsSNPs by structure homology based Method (PolyPhen)

Analyzing the damaged coding non-synonymous SNPs at the structural level are considered to be very important to understand the functional activity of concerned protein. We used the server PolyPhen [14] which is available at http://coot.embl.de/PolyPhen/ for this purpose. Input options for PolyPhen server is protein sequence or SWALL database ID or accession number together with sequence position with two amino acid variants. We submitted the query in the form of protein sequence with mutational position and two amino acid variants. Sequence based characterization of the substitution site, profile analysis of homologous sequences and mapping of substitution site to a known protein 3D structures are the parameters taken into account by PolyPhen server to calculate the score. It calculates position-specific independent counts (PSIC) scores for each of the two variants, and then computes the PSIC scores difference of two variants. Higher the PSIC score difference, higher is the functional impact a particular amino acid substitution is likely to have.

2.4. Scanning of UTR SNPs in UTR site

5′ and 3′ untranslated regions (UTR) of eukaryotic mRNAs are involved in many posttranscriptional regulatory pathways that control mRNA localization, stability and translation efficiency [15,16]. We used the program UTRscan [17] for this analysis. UTRscan looks for UTR functional elements by searching through user-submitted query sequences for the patterns defined in the UTRsite collection.

UTRsite is a collection of functional sequence patterns located in 5′ or 3′ UTR sequences. Briefly, two or three sequences of each UTR SNP that have a different nucleo-

tide at an SNP position are analyzed by UTRscan, which looks for UTR functional elements by searching through user-submitted sequence data for the patterns defined in the UTRsite and UTR databases. If different sequences for each UTR SNP are found to have different functional patterns, this UTR SNP is predicted to have functional significance. The internet resources for UTR analysis are UTRdb and UTRsite. UTRdb contains experimentally proven biological activity of functional patterns of UTR sequence from eukaryotic mRNAs [18]. The UTRsite has the data collected from UTRdb and also is continuously enriched with new functional patterns.

2.5. Modeling nsSNP locations on protein structure and their RMSD difference

Structure analysis was performed for evaluating the structural stability of native and mutant protein. We used the web resource SAAPdb [19] dbSNP [11] to identify the protein coded by ABL1 gene (PDB id 1OPL). We also confirmed the mutation positions and the mutation residues from this server. These mutation positions and residues were in complete agreement with the results obtained with SIFT and PolyPhen programs. The mutation was performed by using SWISSPDB viewer and energy minimization for 3D structures was performed by NOMAD-Ref server [20]. This server use Gromacs as default forcefield for energy minimization based on the methods of steepest descent, conjugate gradient and L-BFGS methods [21]. We used conjugate gradient method for optimizing the 3D structures. Deviation between the two structures is evaluated by their RMSD values.

2.6. Computation of stabilizing residues

In order to check the stability for the native and mutant modeled structures, identification of the stabilizing residues will be useful. We used the server SRide [22] for identifying the stabilizing residues in native protein and in mutant models. Stabilizing residues were computed using the parameters such as surrounding hydrophobicity, long-range order, stabilization center and conservation score as described by Gromiha et al. [22].

3. Results and discussion

3.1. SNP dataset from dbSNP

The ABL1 gene investigated in this work was retrieved from dbSNP database [11]. It contained a total of 827 SNPs. Out of which, 18 were non-synonymous SNPs (nsSNPs), 30 were in non-coding regions, which comprises of 3 SNPs in 5′ UTR region and 27 SNPs in 3′ UTR region. The rest were in the intron region. We selected non-synonymous coding SNPs, 5′ and 3′ UTR region SNPs for our investigation and their distributions are shown in Fig. 1. It can be seen from Fig. 1 that 2.17% of the total SNPs

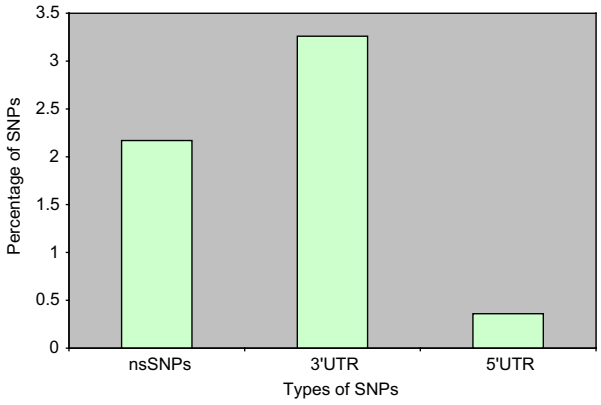

Fig. 1. Distribution of SNPs in non-synonymous, 3′ UTR and 5′ UTR regions.

are nsSNPs, 0.36% and 3.26% of the total SNPs are in 5′ and 3′ UTR regions, respectively. The 5′ UTR region SNPs are much less as compared to both 3′ UTR region SNPs and the SNPs in the coding region. Hence the functional significance due to 5′ UTR region SNPs on the deleterious action of this gene is much less as compared to the SNPs in the 3′ UTR region and the nsSNPs in the coding regions as described in the later part of this work.

3.2. Deleterious nsSNP by SIFT program

The conservation level of a particular position in a protein was determined by using a sequence homology based tool, SIFT [12]. Protein sequences of 18 nsSNPs were submitted independently to SIFT program to check its tolerance index. Higher the tolerance index, less functional impact a particular amino acid substitution is likely to have and *vice-versa*. Among the 18 nsSNPs, 3 nsSNPs were found to be deleterious having the tolerance index score of $\leqslant 0.05$. The results are shown in Table 1.

We observed that, out of 3 deleterious nsSNPs, nsSNPs with an id (rs1064152 and rs1064153) showed a highly deleterious tolerance index score of 0.00 and nsSNPs with an id rs2229071 had a tolerance index score of 0.02. nsSNPs (rs1064152 and rs2229071) had a nucleotide change from C → T and nsSNPs(rs1064153) had a nucleotide change from A → G, respectively.

Table 1
List of nsSNPs that were predicted to be functional significance by SIFT and PolyPhen in ABL1 gene

SNPs ID	Nucleotide change	AA change	Tolerance index	PSIC
rs1064152	C/T	L159P	**0.00**	**2.467**
rs1064153	A/G	G178S	**0.00**	**2.002**
rs1064156	A/G	E478K	0.08	1.753
rs2229071	C/T	P829L	**0.02**	**1.753**
rs11793737	G/T	G862W	0.22	1.675
rs35266696	C/T	P919S	0.29	1.697
rs1064164	C/G	Q986H	0.14	2.052
rs2229067	C/T	S991L	0.10	1.648

nsSNPs scores found to be functional significance by both SIFT and PolyPhen are shown in bold.

3.3. Damaged nsSNP by PolyPhen server

The structural levels of alteration were determined by applying PolyPhen program [14]. All the 18 protein sequences of nsSNPs submitted to SIFT were also submitted as input to the PolyPhen server. Table 1 shows the results obtained from the PolyPhen server. A PSIC score difference of 1.5 and above is considered to be damaging. It can be seen that, out of 18 nsSNPs, 8 nsSNPs were considered to be damaging. All the 8 nsSNPs exhibited a range of PSIC score difference between 1.64 and 2.46.

Among the 3 nsSNPs which were observed to be deleterious by the SIFT program, three of them (rs1064152, rs1064153 and rs2229071) was also found to be damaging according to PolyPhen. Hence, we could infer that the result obtained on the basis of sequence details (SIFT) were in good correlation with the result obtained by structural details (PolyPhen). It can be seen from Table 1 that both the nsSNPs (rs1064152 and rs1064153) had a SIFT tolerance index of 0.0 and for the id (rs2229071) had a score of 0.02. The PSIC score difference are 2.467, 2.002 and 1.797 for (rs1064152, rs1064153 and rs2229071), respectively. Hence one major observation according to SIFT and PolyPhen results is that, the mutation occurring in this nsSNPs would be significant in the identification of chronic myeloid leukemia due to ABL1 gene.

3.4. Functional SNPs in UTR by UTRscan server

Polymorphism in the 3′ UTR region affects the gene expression by affecting the ribosomal translation of mRNA or by influencing the RNA half-life [23]. Table 2 shows the list of SNPs in the 3′ untranslated regions which are predicted to be of functional significance. We used the UTRscan server for this purpose [17]. The UTRscan server finds the patterns of regulatory region motif from UTR database and gives the information about whether the matched pattern is damaged or not. Table 2 shows that out of 27 SNPs in the 3′ UTR region only six SNPs, namely (rs36038391, rs35540520, rs34806832, rs34712944, rs34448359 and rs7850647) were found to have functional significance. 15-Lipoxygenase differentiation control element (15-LOX-DICE) controls 15-LOX synthesis which catalyses the degradation of lipids and is an important factor responsible for the degradation of mitochondria during reticulocyte maturation. This 15-LOX-DICE exist in these 3′ UTR region SNPs which could be considered to be of functional significance in the ABL1 gene.

Table 2
List of SNPs (UTR mRNA) that were predicted to be of functional significance by UTR scan

SNPs ID	Nucleotide change	UTR position	Functional element change
rs36038391	C/T	3′ UTR	15-LOX-DICE → No pattern
rs35540520	A/G	3′ UTR	15-LOX-DICE → No pattern
rs34806832	C/G	3′ UTR	15-LOX-DICE → No pattern
rs34712944	C/G	3′ UTR	15-LOX-DICE → No pattern
rs34448359	A/G	3′ UTR	15-LOX-DICE → No pattern
rs7850647	C/T	3′ UTR	15-LOX-DICE → No pattern

3.5. Modeling of mutant structure

Mapping the deleterious nsSNPs into protein structure information was obtained from Single Amino Acid Polymorphism database (SAAPdb) [19] and dbSNP [11]. The available structure for ABL1 gene is reported to be having a PDB id 1OPL.

According to this resource, the mutations mainly occurred for 1OPL at 2 SNP ids, namely (rs1064152 and rs1064153). The mutation were at the residue position 159 (L → P) and at the position 178 (G → S). The mutation for 1OPL at the position 159 and 178 were performed by SWISS-PDB viewer independently to get two modeled structures. Then, energy minimizations were performed by NOMAD-Ref server [20] for the native type protein (PDB 1OPL) and the two mutant type proteins (1OPL) 159 (L → P) and (1OPL) 178 (G → S).

It can be seen from Table 3 that total energy for the native type structure (PDB 1OPL), and the two mutant type structures 1OPL 159 (L → P) and 1OPL 178 (G → S) were found to be −26743.30, −26721.08 and −2684.05 kcal/mol, respectively. Table 3 also shows that the RMSD values between the native type (1OPL) and the mutant type 1OPL 159 (L → P) is 1.09 Å and between the native type (1OPL) and the mutant type 1OPL 178 (G → S) is 1.13 Å, respectively. Since the RMSD values and total energy after energy minimization are almost similar for both the mutant type structures as compared to the native type structure (1OPL), we may presume that, these mutations do not cause a significant change in the mutant structures of the protein with respect to the native protein structure. However, these mutations could be considered to be functionally significant based on both sequence homology (SIFT), and structural homology method (PolyPhen) results. The native type structure showing leucine residue at position 159 and superimposed structures of the native protein (1OPL)

Table 3
RMSD and total energy of native type protein (1OPL) and mutant proteins

Parameters	1OPL native type structure	1OPL (L159P) mutant model structure	1OPL (G178S) mutant model
RMSD of entire structure		1.09 Å	1.13 Å
Total energy after energy minimization	−26743.305 kcal/mol	−26721.084 kcal/mol	−26894.057 kcal/mol

with mutant type protein 159 (L → P) are shown in Fig. 2(a) and (b), respectively. Similarly, native type structure showing glycine residue at position 178 and superimposed structures of the native protein (1OPL) with mutant type protein 178 (G → S) are shown in Fig. 3(a) and (b), respectively.

3.6. Computing stabilizing residues between native structure and mutant modeled structures

We used SRide server [22] for identifying the stabilizing residues of native type structure and mutant modeled structures. The results are shown in Table 4. Three stabilizing residues, namely, Val6, Ala7 and Trp344 were identified to be common in native type 1X0O structure and in mutant modeled structure 178 (G → S). Out of three stabilizing residues which were identified in native model only two residues (Val6 and Ala7) were seen in mutant model 159 (L → P). From the observation on the overall results on SIFT, PolyPhen and stabilizing residues, we predict that both the mutation 159 (L → P) and 178 (G → S) in the native type protein should be considered to be important mutations in causing chronic myeloid leukemia by ABL1 gene.

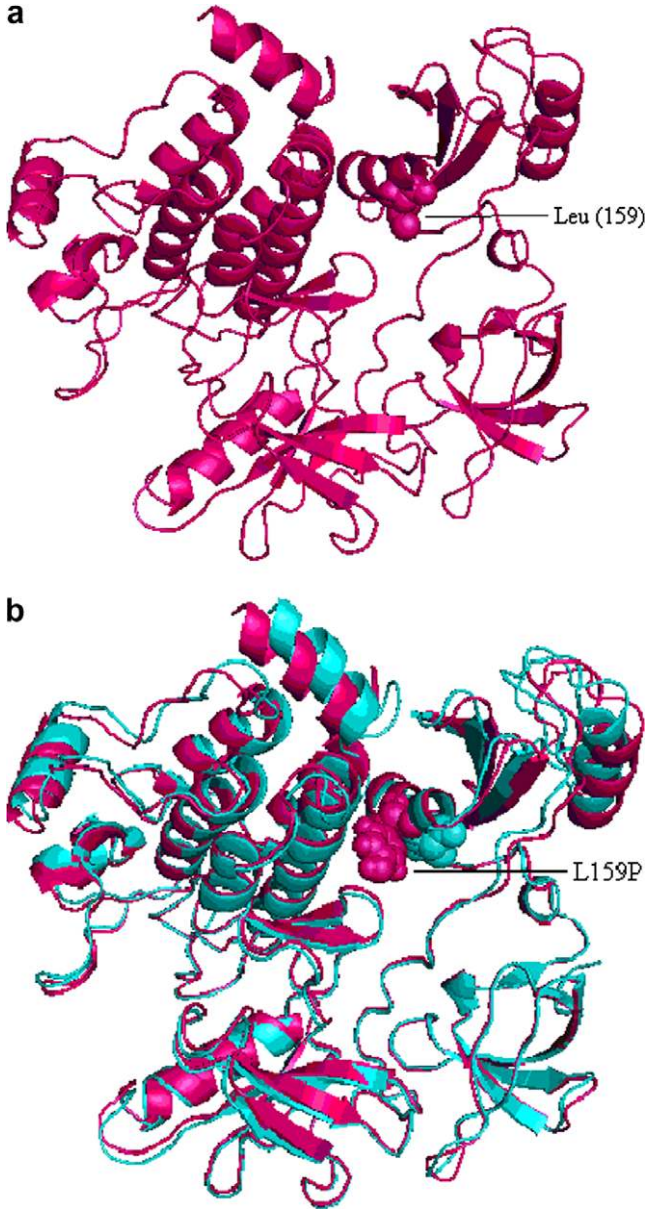

Fig. 2. (a) Native structure of 1OPL showing leucine residue at position 159. (b) Superimposed structure of native protein 1OPL (pink) with mutant structure (cyan) 1OPL 159 (L → P). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

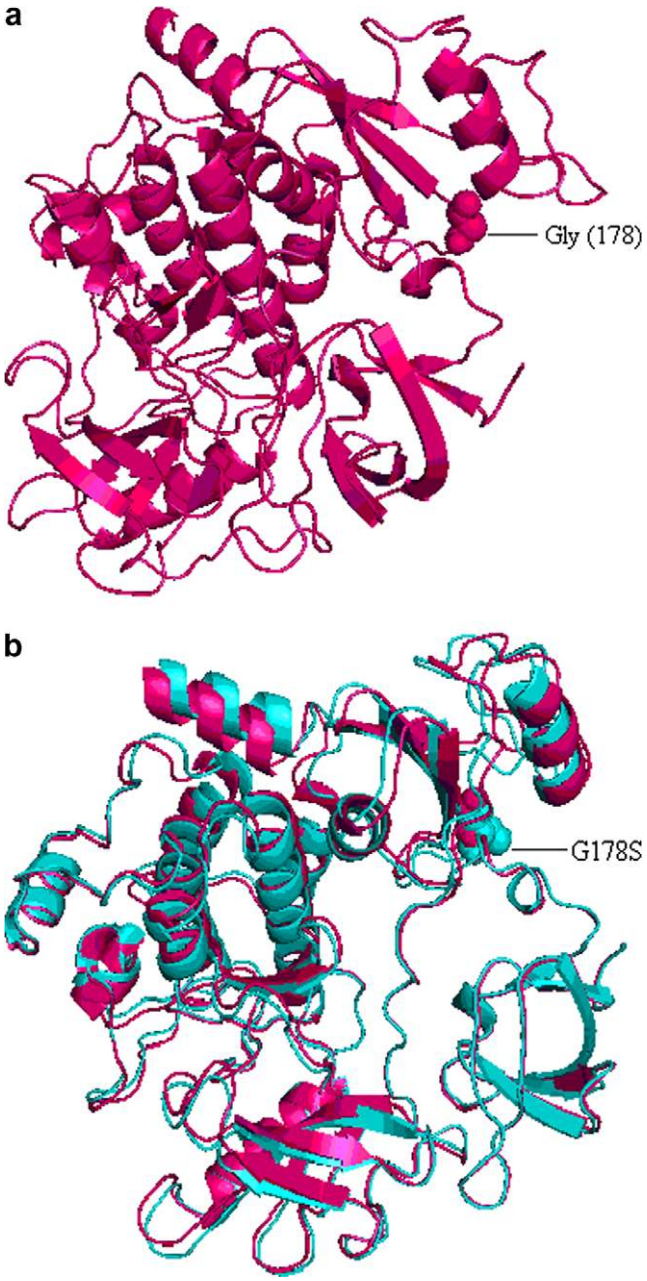

Fig. 3. (a) Native structure of 1OPL showing glycine residue at position 178. (b) Superimposed structure of native protein 1OPL (pink) with mutant structure (cyan) 1OPL 178 (G → S). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Stabilizing residues in native type protein(1OPL) and mutant proteins

Native type structure (1OPL)	Mutant model structure 1OPL (L159P)	Mutant model structure 1OPL (G178S)
Val (6)	**Val (6)**	**Val (6)**
Ala (7)	**Ala (7)**	**Ala (7)**
Trp (344)	Arg (105)	**Trp (344)**

Residues shown in bold are common to both native and mutant protein structures.

4. Conclusion

Structural significance of native and mutant models of chronic myeloid leukemia ABL1 gene was investigated in this work by evaluating the influence of functional SNPs through computational methods. Out of a total of 827 SNPs in ABL1 gene, 18 SNPs were found to be non-synonymous. Three and 27 SNPs were found to be in 5′ and 3′ untranslated regions. Out of 18 nsSNPs, 3 of them were found to be deleterious from SIFT and 8 of them were damaging as per the PolyPhen server. Three nsSNPs were found to be common in both SIFT and PolyPhen server. Six SNPs in the 3′ UTR region were found to be of functional significance. It was found that the major mutations in the native protein of ABL1 gene were from 159 (L → P) and 178 (G → S). We conclude based on the overall results from this study, that both the mutations should be considered important in causing the chronic myeloid leukemia by ABL1 gene. Single nucleotide polymorphisms can be identified by mutation/SNP detection methods including denaturing high-performance liquid chromatography [dHPLC], single-strand conformation polymorphism [SSCP], conformation-sensitive gel electrophoresis [CSGE], chemical cleavage and direct sequencing. This information will help the biologists who are working on ABL1 gene in future.

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