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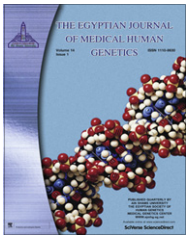

ORIGINAL ARTICLE

Novel mutation predicted to disrupt SGOL1 protein function

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Abstract Cell cycle alterations are the major cause of cancers in human. The proper segregation of sister chromatids during the cell division process defines the fate of daughter cells which is efficiently maintained by various proteomic complexes and signaling cascades. Shugosin (*SGOL1*) is one among those proteins which are required for phosphatise 2A protein (*PP2A*) localization to centromeres during division. This localization actively manages the adherence of sister chromatids at the centromeric region until the checkpoint signals are received. Wide evidences of *SGOL1* genomic variants have been studied for their correlation with chromosomal instability and chromatid segregation errors. Here we used computational methods to prioritize the Single Nucleotide Polymorphism's (SNP's) capable of disrupting the normal functionality of *SGOL1* protein. L54Q, a mutation predicted as deleterious in this study was found to be located in N-terminal coiled coil domain which is effectively involved in the proper localization of *PP2A* to centromere. We further examined the effect of this mutation over the translational efficiency of the SGOL1 coding gene. Our analysis revealed major structural consequences of mutation over folding conformation of the 3rd exon. Further we carried molecular dynamic simulations to unravel the structural variations induced by this mutation in *SGOL1* N-terminal coiled coil domain. Root mean square deviation (RMSD), root mean square fluc-

Abbreviations: nsSNP, non-synonymous single nucleotide polymorphism; dbSNP, database of SNP; Polyphen server, polymorphism phenotype server;
PhD-SNP, predictor of human deleterious single nucleotide; fs, femtosecond; ps, picosecond

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tuation (RMSF), H-Bond scores further supported our result. The result obtained in our study will provide a landmark to future research in understanding genotype-phenotype association of damaging non-synonymous SNPs (nsSNPs) in several other centromere proteins as done in *SGOL1* and will be helpful to forecast their role in chromosomal instabilities and solid tumor formation.

1. Introduction

Aneuploidy has been reported to play a leading role in chromosomal instability (CIN), and is considered as a hallmark of cancer progression [1,2]. The main causal event that leads to aneuploidy are the errors in spindle assembly checkpoint signals and faulty premature segregation of sister chromatids during mitosis. In the case of humans, errors in mitotic chromosomal segregation play a significant role in the onset of neoplasia by reducing tumor suppressor gene dosage or by deregulation of tumor gene activities [3]. To restrain these accidental cellular instabilities, human cells have evolved various molecular pathways, also known as checkpoints [4]. Failure to maintain proper chromosomal segregations and genetic stability during the cell division process will eventually lead to cell death or uncontrolled cell divisions, and could play a detrimental role in inducing cancer [5]. These pathway cascades are regulated by cohesin complex which resists chromatid segregation until the spindle checkpoints are satisfied. Separase cleaves the Cohesin complex by identifying phosphorylated domain of mitotic-sister chromatid cohesion 1 (SCC1) protein, initiating chromatid segregation and thus enables metaphase to anaphase transition [6,7]. Faulty activation of separase activity on mitotic-Scc1 protein initiates premature chromatid segregation, which may lead to aneuploidy and solid tumor formation [8–10]. Cohesin is protected from faulty separase activity by *SGOL1*. *SGOL1* plays an active role in cell division by recruiting *PP2A* to the centrosome [11,12], by its N-terminal 176 amino acids residue. The *SGOL1-PP2A* complex keeps Separase activity in check until all the chromosomes are arranged in their specific order on a spindle microtubule [14,15]. *SGOL1* mediated *PP2A* recruitment process, involving its N-terminal coiled coil domain (residues 47–105) [13] has been reported sensitive to genetic mutations in context to their role in promoting CIN phenotype and aneuploidy. Their role in pathogenicity was further confirmed in the case of colorectal and colon cancers, but little is known about how it is involved [16,17].

Genetic instability in cancers arises either by microsatellite instability (MIN) or by chromosome instability (CIN), which as discussed is the major cause of aneuploidy [18] and has been studied in correlation to solid tumor formation in many cases, and recently was observed in the case of colon cancer [16,17]. The CIN phenotypes are studied with high evidential proximities in solid tumors [19,20]. Experimental evidences strongly support the presence of CIN phenotype at early stages of tumor formation [21–23]. So for in-depth understanding of these physiological and tumorigenic changes in the cell, studies concentrating on the genetic basis of CIN phenotype and their role in tumor formation should be carried out.

SNPs are a class of genetic variations that accounts for single point mutations in the genome and are possibly involved in various disorders and positive adaptations as well. Various point mutations are being extensively studied since several years in correlation to their disease causing potential including deadly diseases such as cancer. The nsSNP's occurring in the coding regions result in single amino acid polymorphisms that may alter the atomic coordinates in protein 3D structure, and has been reported in various oncogenic transformations of the signaling pathways [24]. More than 10 million of SNP datasets have been validated in the NCBI dbSNP [25] database. Most of them are still unclassified in terms of their disease causing potential. By the advancement of sequencing technologies the future of SNP research has greatly risen in the field of personalized medicines and target based drug therapies [26].

Identifying the important candidate genes which are actively participating in chromosomal segregation and their further analysis by mutation induced genotype-phenotype association may prove to be an emergent approach in the field of computational characterization of nsSNP's. We used a set of computational experiments to identify the potential genetic variants which may show the similar oncogenicity in humans. The focal point of this research work is to prioritize most deleterious disease associated nsSNP's (non-synonymous single nucleotide point mutations) in *SGOL1*, on the basis of the evolutionary conservation scores, Support Vector Machine (SVM) based tool and structural simulations of the native and mutant *SGOL1* protein. The SNPs with a very high structural damaging property may lead to a major loss in protein functionality. Conformational changes in the 3D structure of the protein account for its time dependent physiological affinities and various biochemical pathway alterations [27–31]. To examine the molecular and structural basis of predicted disease-associated nsSNPs we then carried out mRNA structure analysis and Molecular Dynamics Simulation.

2. Material and methodology

2.1. Dataset collection

The amino acid sequence [Uniprot: Q5FBB7] of *SGOL1* protein was obtained from UniProt Protein Database [32] and SNP datasets were obtained from NCBI dbSNP database [25] [geneID: 151648]. Structure of *SGOL1* protein N-Terminal Coiled Coil domain [PDB IDs: 3FGA] was retrieved from Protein Data Bank [33].

2.2. Prediction of deleterious and damaging nsSNPs

SIFT algorithm uses a sequence homology-based approach to predict the significant deleterious mutation in the given nsSNP dataset [26]. Prediction is based on the evolutionary conservation of the amino acids within the related protein families [34]. The positions with high conservation score tend to be intolerant to substitution, whereas those with a low degree of conservation tolerate most substitutions. It uses Position Specific Iterative–BLAST (PSI-BLAST) algorithm to analyze the input

sequence by functionally related dataset compilation. Further it scans individual positions of the sequence and calculates the conservation probability of a particular residue for all possible substitutions [35]. SIFT score < 0.05 predicts the substitution as deleterious. Accuracy level of the SIFT program is 88.3–90.6% in terms of their specificity [24]. We applied SIFT algorithm for 28 naturally occurring nsSNP's. Polyphen checks if the amino acid change is occurring at the site that is highly conserved and the variation has any structural correlation with the wild type. It also checks for the annotations in the SWALL database. It uses Coils2 program [36] to predict coiled coil regions and the SignalP program to predict signal peptide regions of the protein sequences [37]. It uses BLAST Algorithm to derive the conservation score of the contig nucleotide and their possible functional roles [38]. The PSIC score difference of 1.5 and above is predicted to show significant structural impact on protein. We selected the nsSNPs that were predicted to be deleterious as well as damaging in SIFT and Polyphen scores to further examine their disease-association probability.

2.3. Prediction of disease-associated nsSNPs

PhD-SNP is an SVM based classifier, trained over a huge set of polymorphic data using supervised training algorithm [39]. The prediction is based on the machine learning approach that determines the pathogenicity of the given mutation [40]. Among the 11 deleterious mutations predicted on the basis of evolutionary conservation analysis, one was reported to be associated with disease causing phenomena. Further to validate the above prediction we used MutPred tool [41]. MutPred is a web application developed to classify an amino acid substitution (AAS) as disease-associated or neutral in humans. In addition, it predicts molecular cause of disease/deleterious AAS [41]. It indirectly exploits the structural and functional data available for functional prediction, effectively enlarging the training data sets beyond the characterized disease-causing events. The output of MutPred contains a general score (g), which defines the probability of amino acid substitution to disease-associated, and top five property scores (p), where p is the P-value on which certain structural and functional properties are impacted. Scores with $g > 0.5$ & $p < 0.05$ are referred to as actionable hypotheses while scores with $g > 0.75$ & $p < 0.05$ are referred to as confident hypotheses and scores with $g > 0.75$ and $p < 0.01$ are referred to as confident hypotheses.

2.4. Structural simulation of exon 3 mRNA

RNAmut package was used to carry the mRNA structural simulation [42]. L54Q mutation corresponds to nucleotide change from T to A at 316 position of SGOL1 gene and is located at exon 3. Nucleotide sequence of SGOL1 exon 3 was obtained from NCBI nucleotide database. Native and mutant nucleotide sequences were subjected to RNAmut package. Changes in free energy values and 3D conformational variations were calculated.

2.5. Molecular Dynamic Simulation

We used Molecular Dynamics Simulation (MDS) technique to understand the structural variations in the mutant protein structure and their role in the functional impact on the protein. This was performed using the GROMACS 4.5.3 [43] package running on a single Intel Duo Core Lenovo machine with 3 GB RAM and running Ubuntu 11.10 package. The 3D structure of the mutant proteins was modeled using swiss-model workspace [44]. Native and mutant structures were subjected to 5000 iteration cycles of energy minimization using OPLS force field. The protein structures were solvated in a cubic 1.0 nm of 216 SPC water molecules. We used $2CL^{-}$ atoms to neutralize the solvation box using genion tool which randomly substitutes the solvent molecules with ions at the most favorable electrostatic potential positions [45]. The electrostatic interactions were resolved by Particle Mesh Ewald method (PME) [45,46]. We used LINCS algorithm [47] to constrain the bond parameters. The position restrain simulation was carried out in order to allow the solvent molecule to enter inside the protein cavity. It allows withstanding the rigorous molecular dynamic simulation and prevents the breakdown of the structure [48]. The native and the mutant protein structures were then simulated for 2 ns with a time step of 2 fs. Temperature was kept constant at 300 K using the Berendsen temperature coupling method [49] with a coupling constant of 0.1 ps, and pressure was kept constant at 1 bar using the Parrinello–Rahman barostat [50] with a coupling constant of 1.0 ps during sampling. Trajectory files were generated using g_rms, g_rmsf and g_hbond program available in Gromacs 4.5.3 as in-built package. The trajectory data were visualized using XMGRACE program.

Table 1 It shows the tolerance score obtained from SIFT server and PSIC value obtained from Polyphen server. rs ID's in bold represent the deleterious alleles predicted by combining both of the above scores.

Rs allele	Amino acid change	Tolerance index	PSIC score
Rs140691683	A31S	0.32	1.226
Rs145234403	P43S	0.04	1.757
Rs150552043	I46V	1	0.312
Rs192601368	**L54Q**	**0**	**2.018**
Rs145242036	**D80H**	**0.04**	**1.988**
Rs183736430	A112T	0.29	0.224
Rs148317477	**M122T**	**0.05**	**2.094**
Rs147441982	**L138S**	**0**	**1.885**
Rs6806241	V171A	0.48	0.983
Rs140685569	S180F	0.06	1.998
Rs141448850	S194G	0.72	0.263
Rs145787431	**D209H**	**0.01**	**1.654**
Rs144488173	V226D	0.01	1.355
Rs141653863	W248R	0.16	3.551
Rs148005116	T264M	0.07	1.091
Rs142110258	**Y309C**	**0.05**	**2.076**
Rs115863450	P321S	1	0.409
Rs9868701	Q322P	0.13	1.066
Rs146606721	K327R	0.45	1.213
Rs144412206	**D377G**	**0.03**	**2.043**
Rs145134729	K387R	0.06	1.252
Rs139053195	I444V	0.87	0.119
Rs139869512	**N446S**	**0**	**1.699**
Rs146108565	V452A	0.46	1.281
Rs181464459	**P487H**	**0**	**2.499**
Rs13060316	K492Q	0	1.477
Rs77194360	R518H	0.3	0.657

3. Results

SIFT server is used for analyzing the evolutionary conservation score of the given nsSNP's. We tested a total of 28 *SGOL1* gene nsSNP's obtained from dnSNP database. This analysis differentiated highly conserved amino acid residues in native protein with those which were found to be lying in comparatively less conserved regions. We observed a set 12 functionally significant nsSNP's (P43S, L54Q, D80H, M122T, L138S, D209H, V226D, Y309C, D377G, N446S, P487H and K492Q) with a tolerance index equal to or less than 0.05 (Table 1). Among these 12 deleterious predicted nsSNP's, L54Q, L138S, N446S, P487H and K492Q were reported to be highly deleterious, with a tolerance index of zero. To validate these results we further used the Polyphen server. Polyphen reported a different set of 12 nsSNP's (P43S, L54Q, D80H, M122T, L138S, D209H, V226D, Y309C, D377G, N446S and P487H) to be deleterious with Position Specific Independent Count (PSIC) score >1.5. Among these 12 damaging SNP's, 6 (L54Q, M122T, W248R, Y309C, D377G and P487H) were reported to be highly deleterious with a PSIC score greater than 2. It was interesting to see 9 mutations that were identified to be deleterious in SIFT were also reported to be damaging by Polyphen server (Table 1). Disease association analysis of predicted deleterious nsSNP's was carried out by PhD-SNP and MutPred tool. PhD-SNP is an SVM based tool with high accuracy of disease allele prediction whereas MutPred, trained on millions of disease association datasets, is the only server that is capable of predicting molecular basis of disease association for given SNP dataset. By blending the results of PhD-SNP and MutPred tools we found L54Q mutation as potentially deleterious and disease-associated (Table 2).

Change in nucleotide position can affect the translational efficiency of the gene. We examined if T316A nucleotide change that corresponds to L54Q mutation, could affect the translational efficiency of SGOL1 gene and so we subjected native and mutant nucleotide sequence of SGOL1 exon 3 to RNAMut package. We observed major free energy loss in mutant mRNA structure. Native mRNA 3D structure contained a free energy value of -30.42 kcal/mol whereas the mutant structure showed a free energy value of -24.94 kcal/mol. This value depicted a major loss in 3D conformation of the mutant SGOL1 exon3 region. The changes in 3D conformation are shown in Fig. 1.

Table 2 The PhD-SNP and Mutpred scores are shown. Results shown in bold represent disease associated allele predicted by combining the results obtained from both the above servers.

Rs allele	Amino acid change	PhD-SNP	MutPred
Rs145234403	P43S	Neutral	0.529
Rs192601368	**L54Q**	**Disease-related**	**0.852**
Rs145242036	D80H	Neutral	0.229
Rs148317477	M122T	Neutral	0.154
Rs147441982	L138S	Neutral	0.232
Rs145787431	D209H	Neutral	0.222
Rs142110258	Y309C	Neutral	0.132
Rs144412206	D377G	Neutral	0.078
Rs139869512	N446S	Neutral	0.394
Rs181464459	P487H	Neutral	0.729

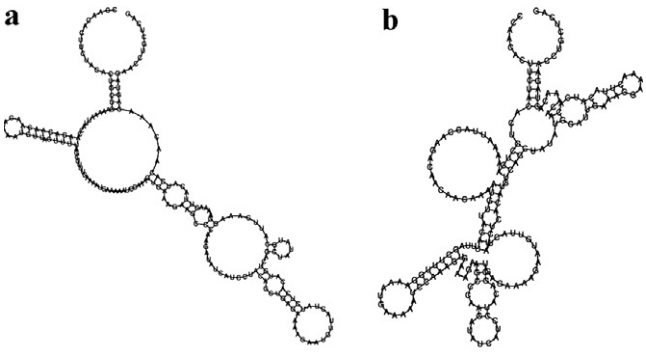

Figure 1 Changes in native and mutant 3D conformation of SGOL1 exon 3 is shown. (a) Native, (b) Mutant.

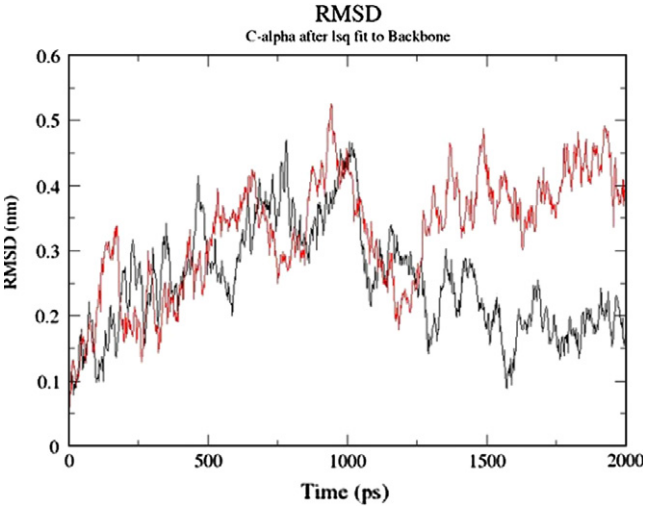

Figure 2 The root mean square deviation plot has been shown. The graph shows higher deviation in mutant structure as compared to the native. Color code for the plot is as follows: black (native) and red (mutant).

Further we used molecular dynamic simulation technique to analyze the impact of mutation over coiled coil motif structure. It was interesting to observe that the mutant RMSD trajectory showed an abrupt rise in RMSD value after 1250 ps time steps and stabilized at 0.4 nm, whereas the native structure stabilized at 0.16 nm (Fig. 2). Also the mutant was found to reach its maximum RMSD score of 0.52 nm at 980 ps whereas the native RMSD reached its highest value of 0.46 at 760 ps. A similar result was observed in RMS distribution plot where the native structure RMS value started from 0.05 nm and reached its highest peak of 0.46 nm whereas the mutant structure RMS value started from 0.05 nm and raised to its highest score of 0.56 nm (Fig. 3). A supportive result was observed when hydrogen bond counts were plotted for 2 ns simulation time scale. Native structure showed a higher number of H-bonds in the loop after 1000 ps as compared to the mutant structure H-bond plot (Fig. 4). H-bonds are considered as vital for maintaining stability in the loop region. It

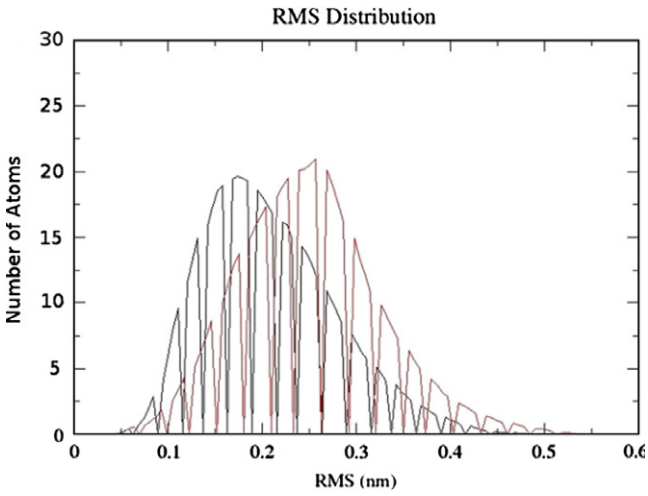

Figure 3 Root mean square fluctuation plot is shown. The graph shows higher atomic fluctuation in mutant structure as compared to the native. Color code for the plot is as follows: black (native) and red (mutant).

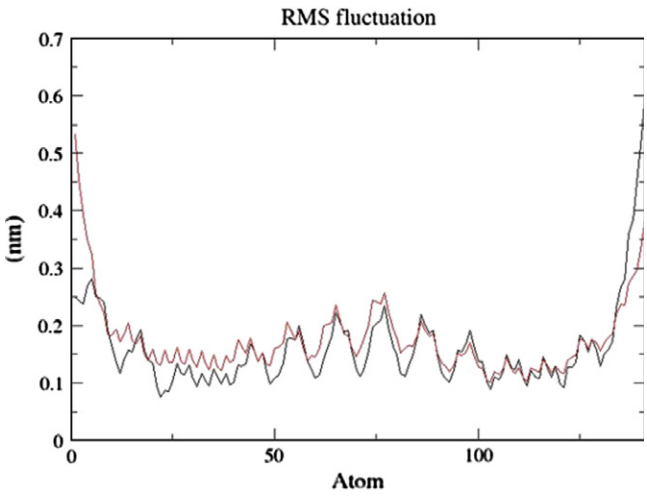

Figure 5 Hydrogen bond plot for 2 ns for native and mutant protein is shown. The graph clearly depicts the loss of H-bond formation in the mutant structure as compared to the native. Color code for the plot is as follows: black (native) and red (mutant).

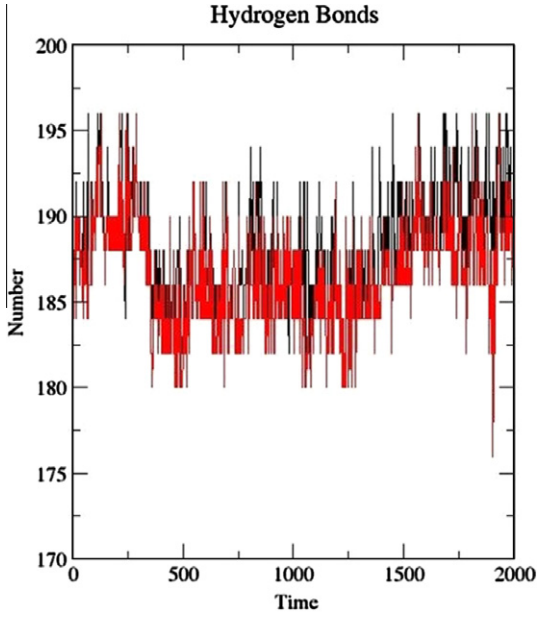

Figure 4 The RMS distribution plot for native and mutant structure is shown. The graph shows higher range of deviation in mutant structure as compared to the native. Color code for the plot is as follows: black (native) and red (mutant).

maintains the angular twist which is important for the stability of coiled helical loops and the loss in H-bond formations may lead to the loss of stability. We investigated the number of H-bond formations in native as well as in mutant structures to examine if the reduction in H-bond has any role in the structural destabilization of mutant protein coiled coil domain. RMSF for all the residues were plotted to crosscheck the notion of mutant protein motif destabilization. RMSF plot was further in high concordance to the results obtained in RMSD and H-bond plots. RMSF value of initial 6 residues (50–55 AA) showed high fluctuations. Later the neighbor residues were also affected by these high terminal fluctuations. All the residues except C-terminal regions were found to be comparatively unstable as observed in the RMSF plot (Fig. 5).

4. Discussion

It has long been recognized that cohesion between sister chromatids in the vicinity of centromeres has an important role during both meiosis and mitosis. Centromeric cohesion is important during cell division because it completely resists pre-mature cell cycle progression and maintains the proper cellular multiplication cascades. The Shugoshin family of proteins plays an important role in the protection of centromeric cohesion [51,52]. In cells with compromised *SGOL1* function, centromeric Cohesin is improperly phosphorylated and removed [53], resulting in premature sister-chromatid separation. There are several other proteins that are involved in maintaining the accuracy of cell cycle checkpoints and thus the cell division process, SGOL1 playing a lead role in this. Several oncogenic and cell cycle checkpoint disorders have been reported in context to the SGOL1 genetic variants and has a leading role in inducing aneuploidy of the cell. By the advancement of sequencing techniques, there has been a huge flooding of polymorphism data in various databases e.g. dbSNP database, and most of the reported polymorphic alleles are still not characterized in terms of their disease association property. Here we used computational techniques to distinguish the potentially damaging genetic variants from those which are pathologically neutral in the SGOL1 coding gene.

Initially we used SIFT, Polyphen, PhD-SNP and Mutpred tools to determine the possible candidate nsSNP with disease-associated phenotypic effects. Previous studies related to deleterious SNP prioritization have been mostly carried out

by evaluating functional and structural significance of given nsSNP datasets. Here we have used functional (SIFT), structural (Polyphen), support vector machine (PhD-SNP) and molecular pathway analysis (MutPred) to maintain a high accuracy level of prediction. We observed high concordance between SIFT and Polyphen results where SIFT predicting total 12 nsSNP as deleterious and Polyphen predicting 12 nsSNP's as damaging has commonly predicting 11 nsSNP's as potentially significant. This observation shows the high accuracy level of prediction carried out by both the tools. Further PhD-SNP and MutPred tools were used to pick out the disease related nsSNP from the selected deleterious nsSNP dataset. We found L54Q as highly deleterious and disease associated, commonly predicted by both these servers. MutPred reported L54Q showing loss of stability ($P = 0.0272$) which was in concordance to the Polyphen result. This mutation was found to be lying in the N-Terminal coiled coil motif (nt-CCM) of the *SGOL1* protein. This motif has been reported as a highly significant region consisting of L64, L68, Y57, N60, E69, K62, L83, K87, Y90, and C94 involved in direct physical interaction with *PP2A* and thus playing a significant role in their localization. Other than this, L53, L54 and I81 are important for dimerization and homodimer stability. Mutations in this domain of *SGOL1* protein have been previously studied for inducing CIN phenotype in mitotically dividing cells. Previously N61I mutation found in the nt-CCM was found to restrict *SGOL1* mediated *PP2A* localization to centrosome [54]. Our result was found to be in concordance to Xu et al. [33] where L54 though not involved in direct interaction with *PP2A* protein, but was found to be highly significant for maintaining the stability of the *SGOL1*a-*SGOL1*b homodimer which is essential for *PP2A* recruitment.

mRNA simulation was performed to investigate the effect of mutation over translational efficiency of the SGOL1 gene. Major loss in free energy and 3D conformation were observed. The mutant conformation of 3rd exon was shown to be disrupted as compared to the native. Further we asked if this mutation has any role in disrupting the nt-CCM structure of the SGOL1 protein. To examine this possibility we carried out Molecular Dynamics Simulation of native and mutant nt-CCM structures using Gromacs 4.5.3 package. A major loss in the stability of mutant L54Q coiled coil motif was observed in RMSD and RMSF plots. In the RMSD plot a sudden rise in structural deviation was observed after 1250 ps, signifying toward its loss of stability. These kinds of changes mostly occur in the case of a major loss of native structural conformations. Coiled coil motifs are especially sensitive to a change in the number of Hydrogen bonds and other stabilizing bonds such as ionic interactions and Vanderwal's contacts. To understand the root cause of the sudden rise in mutant nt-CCM RMSD value, we carried out atomic level investigations by plotting H-bond plots. Interestingly we found a supportive evidence of the loss of H-bond count in mutant nt-CCM after 1 ns timescale. Previous studies have also shown the high importance of leucine in maintaining the native conformation of the coiled loops. The result obtained in this work reports L54Q as a potentially significant mutation, capable of disrupting the N-Terminal Coiled Coil motif of *SGOL1* protein. Although several amino acid residues are not significant as shown in our study but the specific regions that maintain the stability can be involved in damaging the structural conformation of this domain. Alteration in functional mechanism of one protein could significantly affect the functioning of other partner proteins [55]. Thus, the damaging consequences of L54Q mutation on SGOL1 could also hamper the functional mechanism of related cell cycle checkpoint proteins and may create a cascade of altered genomic pathways, ultimately leading to pathological consequences in the cell. The obtained result highlights the effect of a L54Q point mutation over the major structural and functional aspects of *SGOL1* coiled coil nt-CCM and could be a promising candidate for further wet lab analysis to evaluate their pathological significance. The methodology used in this work is user friendly and is easy to use for the beginner. This approach will be helpful in computational analysis of nsSNP's in context to their pathological significance and thus will facilitate future research in personalized medicine and target based drug therapies.

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