In Silico Analysis for Evaluating the Deleterious Nonsynonymous Single Nucleotide Polymorphisms of UBE2C Gene

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Abstract **- UBE2C encodes a member of E2 ubiquitin conjugating enzyme family involved in Ubiquitination one of the main post-translational modification of proteins. UBE2C is also a key regulator of cell cycle progression and its altered expression is implicated in various malignancies. Now a days UBE2C mutation is studied frequently in number of cancers but before planning large population study, it is better to scrutinize putative functional SNPs of UBE2C gene and its functional partners by using different computational tools. In this study, we have used various computational approaches including PROVEAN, PolyPhen-2, and i-Mutant2.0 tools to identify deleterious SNPs of UBE2C protein and also studied its protein network using STRING database. The present study concluded that 13 nsSNPs: W165R, W62C, P86H, P90A, G111D, L115P, T100M, N106S, K80N, Q136R, L59F, I131M and V107M which were predicted to be highly deleterious and functionally significant nsSNPs in human UBE2C gene.**

Index Terms **- nsSNP, PhD-SNP, PolyPhen-2, PROVEAN, SIFT, UBE2C.**

INTRODUCTION

Ubiquitin conjugating enzyme 2C (E2) in human is encoded by the gene, UBE2C gene located on chromosome location 20q13.12. UBE2C gene has eight transcript variants through alternative splicing. The full length UBE2C contains 179 amino acids with molecular weight of 19.65 kDa (Lin et al., 2002; Townsley et al., 1997). The UBE2C protein is a α/β protein. Its structure contains four stranded antiparallel β sheets (B1-4), a conserved 310-helix (310) and four α-helices (H1-4) (Jiang and Basavappa, 1999). E2s are classified into four classes. All four classes share a conserved core domain containing the catalytic Cys residue. UBE2C is a class 3 E2 enzyme has N-terminal extension from the core domain. The E2 core domain has catalytic Cys114 (located between H4 and 310) which is responsible for ubiquitin adduct formation while the UBE2C N-terminal extension is contributes to the regulation of APC/C activity as a part of inhibitory mechanism (Lin et al., 2002; Wing et al., 1995).

UBE2C involved in ubiquitination one of the main post-translational modification of proteins play role in various cellular functions such as protein degradation (mainly short-lived proteins), protein interactions and subcellular locations. Ubiquitination precisely regulates cell cycle at key check points by targeting cell cycle regulator for proteasome mediated degradation (Dastsooz et al., 2019; Lin et al., 2002). The human UBE2C is functional partner of anaphase promoting complex/cyclosome (APC/C), involved in the degradation of family of APC/C target proteins by initiating Lys11 linked ubiquitin chains (Jin et al., 2008; Meyer & Rape, 2011). UBE2C is also required for the degradation of mitotic cyclins and other mitosis related substrates via APC/CCDC20 to promote the cell cycle progression to anaphase. UBE2C degrades the securing to activate the separase and thus directly promotes the anaphase onset (Aristarkhov et al., 1996; Arvand et al., 1998; Rape & Kirschner, 2004; Rape et al., 2006). This enzyme is also participating in the regulation of spindle assembly checkpoint (SAC) by catalyzing the dissociation of mitotic spindle checkpoint components (MAD2, BUB3, and BubR1) from the APC/C and by antagonizing the deubiquitinating activity of USP44 (Reddy et al., 2007; Stegmeier et al., 2007; Williamson et al., 2011). In addition, UBE2C is also plays a vital role in

maintaining the euploidy status of cells (Van Ree et al., 2010).

Mutation in UBE2C may leads to abnormal enzyme function. UBE2C has a rate-limiting role in the late G1 phase. In late G1 phase UBE2C required to destabilize cyclin A and prevent premature DNA replication (Walker et al, 2008). So, its altered expression due to mutation may lead to pathological consequences. Some studies indicate that the UBE2C possesses oncogenic properties. UBE2C is expressed at relatively low levels in normal tissues whereas the amplification of UBE2C gene has been reported in various malignancies. One study shows that HeLa Cells overexpressing UBE2C enter into mitosis but are failed to maintain spindle checkpoint activity and most of the interphase cells overexpressing UBE2C were multinucleated (Reddy et al, 2007). In humans upregulation of UBE2C have been found in cancers in the Lung (Okamoto et al., 2003; Van Ree et al., 2010), Breast (Wagner et al., 2004; Loussouarn et al., 2009), Ovary (Berlingieri et al., 2007), Uterus (Okamoto et al., 2003), Bladder (Wagner et al., 2004), Gastric (Wagner et al., 2004), Colon (Chen et al., 2010), Liver (Ieta et al., 2007), Thyroid (Pallante et al., 2005) and Brain (Jiang et al., 2008).

Mutations affecting the normal expression of UBE2C include SNPs (Single nucleotide polymorphism), insertions and deletions (Cargill et al., 1999). Reports suggested that 90% of human genetic polymorphism occurs due to SNPs among of which nonsynonymous SNPs in the coding region play a vital role in most of the biological variations and half of them associated with inherited human diseases (Chakravarti A., 2001; Collins et al., 1998; Stenson et al., 2003). The nsSNPs in coding region can have the major impact on the phenotype as it leads to change in the physiochemical property of the native amino acid, which may affect the dynamics and stability of protein and may disrupt the protein-protein and protein-cofactor binding ability (Carninci et al., 2005; Kono et al., 2008; Stitziel et al., 2004; Uzun et al., 2007; Yue and Moult, 2006). A number of genetic variations including SNPs are reported by means of high throughput human genome research (Cargill et al., 1999; Hinds et al., 2005). It is not necessary that all the reported SNPs were deleterious and affect protein structure and function. So, it is important to understand the mechanism of effect of variation on protein stability, structure and function. Phenotypic effect and biochemical severity of amino acid substitution can be understood by in silico approach. More sophisticated in silico tools have been developed, which helps in the prediction of effect of every single amino acid change on protein structure function and stability (Doss et al., 2013; Desai and Chauhan, 2017).

Till date deleterious nsSNPs of human UBE2C gene have not been predicted using in silico analysis. Hence the present study explored the understanding of the association between the genetic variations and phenotypic effect using the in-silico analysis. The current study was undertaken to determine the most deleterious nsSNPs of human UBE2C gene by using PROVEAN, i-Mutant2.0, and PolyPhen-2, PhD-SNP, and SIFT. The deleterious effect of nsSNPs on protein function is analyzed by PROVEAN, PolyPhen-2, and SIFT. PhD-SNP was used for the prediction of disease associated nsSNPs while the impact of nsSNPs on the stability of protein is determined by i-Mutant2.0. String analysis was performed to know protein-protein interactions.

MATERIAL AND METHODS

SNP retrieval:

The data on human UBE2C SNPs (rsIDs), retrieved from dbSNP (NCBI) database (https://www.ncbi.nlm.nih.gov/). The single nucleotide polymorphism database (dbSNP) is free public archive for large collection of genetic polymorphisms includes single base substitution or SNPs, deletion insertion polymorphism or DIPs and reteroposable element insertions and microsatellite repeat variations or STRs (Sherry et al., 2001). The FASTA sequence of the UBE2C gene was obtained from the Uniprot database (https://www.uniprot.org).

Prediction of deleterious nsSNPs of UBE2C gene:

PROVEAN: PROVEAN (Protein Variation Effect Analyzer http://provean.jcvi.org) uses alignment-based score approach to predict functional effect of single or multiple amino acid substitutions, insertions and deletions. The web server contributes to three functions: PROVEAN protein for any organism, PROVEAN protein batch (human and mouse), PROVEAN genome variants (human and mouse) (Choi et al., 2015). The PROVEAN tool was applied to the dataset to generate a PROVEAN score for each variant. The current default score threshold set at -2.5 for binary classification. If the PROVEAN score is

equal to or below the predefined threshold, the protein variant is predicted to have a "deleterious" effect and if the score is above the predefined threshold, then the variant is predicted to have a "neutral" effect.

PolyPhen-2: PolyPhen-2 (Polymorphism Phenotyping 2 http://genetics.bwh.harvard.edu/pph2/) is an automatic tool for the prediction of possible impact of an amino acid substitution on the structure and function of human proteins. Prediction is based on number of features comprising sequence, phylogenetic and structural information characterizing the substitution. PolyPhen-2 score is ranging from 0.0 (Benign) to 1.0 (Damaging) and prediction outcome can be one of Probably Damaging, Possibly Damaging and Benign (Adzhubei et al., 2013).

SIFT: Sorting Intolerant from Tolerant (SIFT) uses sequence homology to predict the effect of coding variant on protein function. It was first introduced in 2001 and has become one of the standard tools for characterising missense variations. Web server of SIFT hosted by the original developers at FHCRC and JCVI currently located at BII. SIFT is freely available to all users at https://sift.bii.a-star.edu.sg/. Prediction is classified between Tolerated and Affect protein function. The value of threshold score is set at 0.05. If the SIFT score is equal to or below the predefined threshold, substitution is predicted as "Affect Protein Function" and if the SIFT score is above the predefined threshold, then substitution is predicted as "Tolerated" (Sim et al., 2012).

Prediction of disease associated SNPs:

PhD-SNP: PhD-SNP is based on Support Vector Machine (SVM) method. This tool is available via a web server (http://snps.biofold.org/phd-snp) to predict the effect of amino acid substitution on protein function and structure. SVM classifies the prediction into disease related (desired output set to 0) and neutral polymorphism (desired output set to 1). The threshold value is set to 0.5. PhD-SNP required a protein sequence as input and the position number in the sequence of the residue undergoes mutation. Prediction is choosing between the sequence based and profile-based prediction. Output of PhD-SNP consist a table listing the number mutated position of protein sequence, the wild type of residue, the new residue, RI (Reliability Index) value and if the related mutation is predicted as disease related (Disease) and neutral polymorphism (Neutral).

Prediction of change in the protein stability:

I-Mutant2.0: The impact of all single nucleotide variations of human UBE2C gene on protein stability was analyzed using tool i-Mutant2.0 (https://folding.biofold.org/i-mutant/). I-Mutant2.0 is a Support Vector Machine (SVM) based web server which predicts the variance in stability occurring due to point mutation through neural network algorithm. It is used for automatic prediction of protein stability changes upon site mutation. Prediction can be starting either from protein structure or from protein sequence. This method was trained and tested on a data set derived from most comprehensive available database ProTherm. This tool can be used both as a classifier and regression estimator for predicting the sign of the protein stability change and DDG value. Depend upon the structure and sequence-based prediction expected value (0.71 and 0.62) is taken from experimental database to predict the correlation when the predicting DDG value is associated with the mutation (Capriotti et al., 2005).

Protein-Protein Interaction Prediction:

The interactions of UBE2C with other proteins were predicted using STRING (Search tool for the retrieval of interacting proteins, https://string-db.org/). The STRING predicts protein-protein interaction by means of, either direct or indirect, association among a known protein and other protein by utilizing its database of 5,214,234 proteins of 1113 (Szklarczyk et al., 2011). For STRING prediction UBE2C and Homo sapiens were used as input.

RESULTS

SNPs Retrieval:

The NCBI-dbSNP showed a total of 2105 SNPs reported in human UBE2C gene. Out of which only 62 missense variants were further used for analysis.

Table 1) List of SNPs retrieved from dbSNP

Sr.No	rsNumber	Amino Acid Change
1	rs7352110	R129G
2	rs11537645	S23R
3	rs61760191	N158K
4	rs138058971	M40V
5	rs1402378082	D145N
6	rs144831911	D57N
7	rs145980514	G88S
8	rs148799246	Y126C

9	rs201125748	E154K
10	rs201176918	I131M
11	rs202149476	T169A
12	rs367706641	T95A
13	rs371311367	T130N
14	rs371616231	P86H
15	rs373084608	T160I
16	rs374439898	P90A
17	rs376878469	V174G
18	rs545175408	M43T
19	rs547850953	R78S
20	rs561525053	R6S
21	rs748461028	S87T
22	rs749925974	A27T
23	rs751722028	E84D
24	rs752378234	A161T
25	rs752601468	G64A
26	rs753288094	S87G
27	rs753350058	Q167E
28	rs756640882	S87R
29	Rs757572822	L125Q
30	rs758135325	N92S
31	rs75857409	T175P
32	rs759551578	P86T
33	rs759589512	L59F
34	rs759764860	V107M
35	rs761307294	L115P
36	rs761413591	G24R
37	rs761500077	Q36E
38	rs761667583	Y79F
39	rs762275535	K157E
40	rs763209065	S82L
41	rs763391914	P159T
42	rs763421388	A10T
43	rs764691584	K121T
44	rs766016081	D47N
45	rs766325761	Q177R
46	rs766882355	A14T
47	rs767520518	G111D
48	rs767562250	W62C
49	rs767584548	L133F
50	rs768695868	E75D
51	rs769693911	K97N
52	rs770303252	K157R
53	rs770704295	N106S
54	rs772939191	K80N
55	rs773029038	T100M
56	rs774646356	R17G
57	rs774676127	A161G
58	rs776802939	W156R
59	rs777703076	Q136R
60	rs778333049	Y89S
61	rs779653538	V73L
62	rs780709357	V96M

Prediction of functional nsSNPs in UBE2C

The UBE2C single nucleotide variants (62) obtained from dbSNP analysis were subjected to computational analysis with the help of variety of tools. Out of total 62 nsSNPs of UBE2C gene, 35 (56.45%) found to be deleterious by PROVEAN and PolyPhen-2 server predicted 19 (30.64%) nsSNPs as probably damaging, 8 (12.90%) as possibly damaging and remaining 35 (56.45%) as benign. Further SIFT tool predicts 22 (35.48%) SNPs affect protein function as the SIFT score is below or equal to 0.05 and remaining 41 (64.51%) SNPs were found to be tolerated as the SIFT score is greater than 0.05. All the 62 nsSNPs of UBE2C gene were further analyzed through PhD-SNP. PhD-SNP is a SVM based classifier which predicts the result through evolutionary information. PhD-SNP depict 20 (32.25%) SNPs are disease associated while remaining 42 (67.74%) SNPs are neutral. Furthermore I-Mutant was used to predict change in protein stability. Out of 62 nsSNPs of UBE2C gene subjected for stability prediction, 50 (80.64%) showed decrease in stability and rest 12 showed increase in protein stability by I-Mutant. Further sorting of nsSNPs according to their energy values indicating only 13 SNPs were found to be extremely deleterious nsSNPs (Table 2).

Table 2) Sorting of deleterious nsSNPs:

Sr. No	rsNumber	A.A Change	PROVEAN		PolyPhen-2		SIFT		PhD-SNP		I-Mutant2.0		
			Score	Prediction	Score	Prediction	Score	Prediction	RI	Prediction	RI	Prediction	DDG value
1	rs776802939	W156R	-13.83	D	0.969	PRD	0	A	4	DI	0	DE	0.11
2	rs767562250	W62C	-12.547	D	1	PRD	0	A	7	DI	3	DE	-1.56
3	rs371616231	P86H	-8.347	D	0.958	PRD	0.01	A	4	DI	0	DE	-0.19

4	rs374439898	P90A	-7.428	D	0.988	PRD	0	A	3	DI	9	DE	-2.41
5	rs767520518	G111D	-6.663	D	1	PRD	0	A	9	DI	5	DE	-1.08
6	rs761307294	L115P	-6.497	D	1	PRD	0	A	6	DI	1	I	-1.36
7	rs773029038	T100M	-5.524	D	1	PRD	0	A	2	DI	4	DE	-1.45
8	rs770704295	N106S	-4.741	D	0.769	PSD	0	A	1	DI	6	DE	-1.2
9	rs772939191	K80N	-4.465	D	0.867	PSD	0	A	1	DI	5	DE	-0.92
10	rs777703076	Q136R	-3.883	D	0.979	PRD	0.02	A	4	DI	7	DE	-1.23
11	rs759589512	L59F	-2.827	D	0.745	PSD	0.02	A	7	DI	1	DE	0.41
12	rs201176918	I131M	-2.535	D	0.982	PRD	0	A	3	DI	6	I	0.87
13	rs759764860	V107M	-2.527	D	1	PRD	0	A	2	DI	9	DE	-2.9

Where D: Deleterious; N: Neutral; DE: Decrease; I: Increase; B: Benign; PRD: Probably Damaging; PSD: Possibly Damaging; DI: Disease; T: Tolerated; A: Affect Protein Function

Protein-Protein Interaction Prediction:

STRING results predicted the functional association of Ubiquitin conjugating enzyme 2C (E2) with CDK1 (Cyclin-dependent kinase 1), CDC20 (Cell division cycle protein 20 homolog), MAD2L1 (Mitotic spindle assembly check point protein MAD2A), PTTG1 (Securin regulatory protein), AURKA (Aurora kinase A), CCNA2 (Cyclin-A2 which control both G1/S and G2/M phase transition), UBE3D (E3 ubiquitin-protein ligase E3D), CCNB1 (G2/Mitotic specific cyclin-B1), CDC16 (Cell division cycle protein 16 homolog) and ANAPC11 (Anaphase promoting complex subunit 11) (Fig.1, table 3).

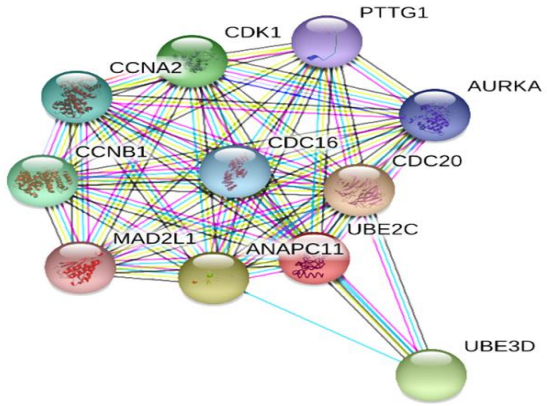

Figure 1: UBE2C protein-protein interaction network shown by STRING.

Table 3). List of the functional partners of ube2c protein

Sr. No	Functional Partners	Description	Coexpression	Experiment	Database	Text mining	Score
1.	CDC20	Cell division cycle protein 20 homolog	Yes	Yes	Yes	Yes	0.999
2.	ANAPC11	Anaphase promoting complex subunit 11	Yes	Yes	Yes	Yes	0.999
3.	UBE3D	E3 ubiquitin-protein ligase E3D	Yes	Yes	Yes	Yes	0.999
4.	CDK1	Cyclin-dependent kinase 1	Yes	Yes	Yes	Yes	0.999
5.	CCNB1	G2/Mitotic specific cyclin-B1	Yes	Yes	Yes	Yes	0.998
6.	CCNA2	Cyclin-A2 which control both G1/S and G2/M phase transition	Yes	Yes	Yes	Yes	0.998
7.	CDC16	Cell devision cycle protein 16 homolog	Yes	Yes	Yes	Yes	0.998
8.	AURKA	Aurora kinase A	Yes	Yes	Yes	Yes	0.998
9.	PTTG1	Securin, regulatory protein	Yes	Yes	Yes	Yes	0.997
10.	MAD2L1	Mitotic spindle assembly checkpoint protein MAD2A	Yes	Yes	Yes	Yes	0.997

DISCUSSION

The UBE2C gene encodes a member of E2 ubiquitine conjugating enzyme family involved in Ubiquitination system in collaboration with APC/C. It is involved in the degradation of mitotic cyclin B and other mitosis related substrate, promoting the transition from the M phase to G1 phase of cell cycle. Therefore, it is likely that mutation in UBE2C gene leads to abnormal enzyme function that is leading to abnormal enzyme function that is leading to changes in ubiquitination, might be involved in uncontrolled cell proliferation, which is one of the main features of malignancies. Among the mutations affecting enzyme function, nsSNPs are very frequently occurring and mostly associated with inherited disorders.

To the date more than 2000 SNPs are reported in human UBE2C gene in NCBI-dbSNP, all may not have deleterious impact on protein function or structure. Non-Synonymous SNPs are the most common form of mutation which affects the proteins biological function by altering the amino acids of the encoded protein. Most of the nsSNPs of human UBE2C gene are uncharacterized for their potential to cause disease. Hence, the current study shows, for the first time, a computational analysis of nsSNPs of UBE2C gene. In this study, we have used various computational approaches to identify the nsSNPs deleterious to structure and/or function of UBE2C protein. The approaches used in current study provide the clues on the effect of variation at molecular level by means of the variation aspects as well as the parameters describing the pathogenicity of amino acid substitution. In present study, three different tools PROVEAN, PolyPhen and SIFT were used to predict the functional effect of nsSNPs; PhD-SNP was used to predict disease associated nsSNPs and as the protein stability is essential for structural and fuctional activity of protein, i-Mutant2.0 tool was used to obtain the deleterious nsSNPs that may affect the protein stability of the UBE2C protein.

Among the 62 nsSNPs found in NCBI database, we finally screened out 13 highly deleterious nsSNPs (W165R, W62C, P86H, P90A, G111D, L115P, T100M, N106S, K80N, Q136R, L59F, I131M and V107M) by comparing the output of five pathogenecity prediction tools (table 2). Moreover, our study recognized co-expression genes related with UBE2C protein network. These include CDC20; UBE3D; ANAPC11; CDK1; CCNB1; CCNA2; CDC16; AURKA; PTTG1; MAD2L1 (Fig 1, table 3). These results may be helpful for further understanding of UBE2C SNPs in disease susceptibility by laboratory experiments.

CONCLUSION

Present study on the computational analysis of functional SNPs of UBE2C provides significant insight into deleterious effect that the nsSNPs cause to the protein. This is the first computational study which predicts the impact of nsSNPs on the structure and function of UBE2C gene. Our study concludes that 13 nsSNPs W165R, W62C, P86H, P90A, G111D, L115P, T100M, N106S, K80N, Q136R, L59F, I131M and V107M were predicted to be highly deleterious among the reported UBE2C gene nsSNPs. All the 13 nsSNPs predicted disease associated as well as pathological and also predicted to affect protein function and stability. Since this gene has been linked to a variety of cancers, our discovery will be useful in future studies of possible diagnostic and therapeutic treatments, as well as experimental research such as drug target prediction and drug design.

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