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Elucidation of the effects of mutations on REP region of PARKIN during the onset of Parkinson's disease

Analysis of Parkin domain mutations

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Abstract*—Autosomal-recessive juvenile parkinsonism (ARJP) is caused due to the mutation in parkin gene and was first reported by kitada et al. in 1998. More than hundreds of mutations are reported in parkin protein which is directly linked to familial recessive form of Parkinson's disease. Human parkin protein is 465 amino-acid long, and located on chromosome 6. The N-terminal ubiquitin-like domain (Ubl) domain and C-terminal four zinc-coordinating cysteine-histidine rich RING like domains: RING0, RING1, IBR and RING2 are the structural features of parkin protein in terms of the distribution of domains. Along with neuroprotective protein PINK1, parkin is also involved in the mitochondrial quality control pathway. Dysfunctions of both the proteins lead to ARJP. Parkin is an E3 ligase and its activity is tightly controlled by multiple mechanisms. A repressor region called REP region is present in between parkin C-terminal IBR and RING2 domains which also controls the parkin activity. This REP region is an all-alpha domain. E3 ligase activity of parkin is suppressed by mutations in RING0 domain and REP region. In mutant parkin, RING0 domain blocks the catalytic residues of RING2 and Ubl and REP blocks the E2 binding region of RING1. It has been shown that E2 binding affinity and parkin activity are increased by deletion of Ubl and REP regions. Various mutations are also reported in REP region that are linked to ARJP. In this work, we first collected a total of 20 mutations in the REP region from literature and different databases. We then analyzed the various features of the mutations in the parkin REP region by using various computational tools like Polyphen2, SNAP2, Aline GV-GD, Proven, SNPs&GO to predict their severity and impact on the protein structure and function. Among the mutations, we identified the five most deleterious mutations, viz., R402G, E404K, S407P, S407C, and S407F. These mutations are considered to be responsible for ARJP onset. This is the first report on the mutations in the REP region of parkin.

***Keywords*— ARJP, Parkin, REP region, PINK1, Mutation.**

INTRODUCTION

Neurodegenerative diseases are responsible for major threats to human health. In recent years, there is an increase in age-related neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, and Huntington's disease in the whole world. Parkinson's disease is the second most common neurodegenerative disorder. Approximately 6.3 million people suffer from Parkinson's disease (PD) worldwide. James Parkinson first coined the term Parkinson Disease in 1817 in his paper entitled "An Essay on the Shaking Palsy" [1]. Motor disorder, bradykinesia, rigidity, postural instability, and tremor are some clinical characteristic features of PD. Deposition of an abnormal protein called Lewy bodies (LW) in the brain cells [2] and degradation of dopamine producing dopaminergic nerve cell located in the substantia-nigra pars compacta [3] are the pathological hallmarks of PD patient. Parkinson disease can be autosomal dominant or sporadic caused by dysfunctions of PARK1/4, PARK8 genes and dysfunction of SNCA, parkin, UCHL1, PINK1, DJ1, and LRRK2 genes are linked to autosomal recessive or familial parkinsonism [4]. More than 150 mutations are spread throughout the parkin protein. In Japan, first parkin mutation was identified which was linked to Autosomal Recessive Juvenile Parkinsonism (ARJP) [5]. Surprisingly, parkin mutations are also linked to melanoma of lung, pancreatic and breast [6, 7, 8, 9]

Mutation in PARK2 (parkin) and PARK6 (PINK1) genes are responsible and directly linked to the ARJP. Both the gene products, viz., parkin and PINK1 proteins, control mitochondrial quality by a cascade mechanism. Human parkin protein contains ubiquitin-like (Ubl) domain located at its N-terminal end and its C-terminal end consist of RING1-IBR-RING2 (RBR) domain [10]. A linker region is present in between the N-terminal Ubl and Zinc ion containing RING0 domain. Another domain Repressor Element of Parkin (REP) is present between the IBR and RING2 domains. Parkin is a RBR E3 ligase and like other E3 ligases participates in the ubiquitination pathway to degrade and remove abnormal proteins from the brain cell. Structural studies of parkin reveal that in inactivated state it exists in a close complex form. In between RING1 and RING2, RING0 domain is inserted in an auto-inhibited state. REP also enhances the close complex

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formation and hides the E2 binding site located in the RING1 domain and thereby preventing E2 protein recruitment. When parkin protein is activated structural rearrangements take place between the domains. Dissociation of RING0 from RING2 and REP from RING1 events occur during this condition. In the active state, first phosphorylation of serine 65 (S65) in the Ubl domain of parkin protein occurs by PINK1 protein. Subsequently, RING1 phosphorylation and others structural changes happen.

REP region plays a crucial role into maintaining parkin protein's close complex structure and E2 subunit binding. Mutation in this region may hamper the protein's normal structure and function. In this present work, we are trying to analyze the mutational effects to identify the most deleterious mutations. This in-silico study may shed light to understand the structural and functional changes in the REP region of parkin during the onset of PD. due to the mutations and help scientists to identify novel therapeutics.

MATERIALS AND METHODS

Data collection: Mis-sense mutational data were retrieved using PDmutDB database and from the literature. The protein sequence information of PARK2 (UniProtKB id- O60260) was extracted from UniProt database. The protein sequence is required for various analyses to predict the effects of the mutations.

In-silico methods to predict mutational effect: We used the available different computational tools to analyze twenty mis-sense mutations in the REP region of parkin. We used SNPs & GO (https://snps-andgo.biocomp.unibo.it/snps-and-go/)[11] which is based on the principle of Support Vector Machine (SVM) to predict the disease related missense mutations. The results were retrieved as disease-related or neutral. We used PolyPhen-2 (Polymorphism Phenotyping v2) program (http://genetics.bwh.harvard.edu/pph2/) [12] for analysis of the mutations as well. PolyPhen-2 uses structural and comparative evolutionary considerations to predict the impacts of possible amino acid substitutions on human protein stability and function by providing scores. PROVEAN (Protein Variation Effect Analyzer) (http://provean.jcvi.org/) predicts the amino acid substitution as either neutral or deleterious [13, 14]. We analyzed the mutations using PROVEAN as well. Based on the evolutionary information SNAP2 (screening for non-acceptable polymorphisms) program (www.rostlab.org/services/SNAP/) predicts the mutational effects on the protein [15]. We analyzed the mutations using SNAP2. Align-GV-GD (http://agvgd.iarc.fr/agvgd) classifies variants as "neutral," "unclassified", or "deleterious" by using combination of Grantham Variation (GV) and Grantham Deviation (GD) algorithm. This tool was also used to analyze the mutations. We used all these tools to analyze the effects of the mutations in order to come up with a consensus result. I-Mutant 2.0 (http://folding.biofold.org/i-mutant/i-mutant2.0) and Mupro (http://mupro.proteomics.ics.uci.edu/) online tools are used to predict protein stability. By using five independent algorithms SOPMA server (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_ sopma.html) analyses the secondary structure of proteins. We used these two aforementioned tools to identify the secondary structural distributions in the REP region before and after mutation.

RESULT AND DISCUSSION

Our aim of this work is to predict the mutational effect on the REP region of parkin protein. REP region plays a crucial role in the activation of parkin protein as well as E2 protein binding to parkin. Mutation in this region may be responsible for the onset of ARJP and due to the mutations protein structure and its function are hampered. Using different computational tools the different missense mutations in the REP region of parkin protein were analyzed. First, all mutations were analyzed using five bioinformatics tools, viz., SNPs&GO, Polyphen2, PROVEAN, SNAP2, and Align-GV-GD tools. Table1 reflects the mutational prediction results. All these software tools use different algorithms to produce the results. SNPs&GO identified 3 mutations R402G, S407P and S407F to be disease causing. PolyPhen-2 predicted 8 mutations to be benign, 3 to be possibly damaging, and 9 to be probably damaging. Seven mis-sense mutations were predicted to be deleterious by PROVEAN server. SNAP2 reported nine mutations to be pathogenic and affect the protein structure. We scored the mutations on a statistical basis and marked R402G, E404K, S407P, S407C, and S407F as the most pathogenic mutations (table 1). We also checked the protein stability change due to the mutations and analyzed the secondary structural distributions.

Secondary structure of this region was predicted by SOPMA online tools. Mutations in the core region and surface region of the protein may cause harmful effects on its secondary structure and sometime inhibit the interactions with other proteins. So, it is necessary to identify weather the mutation is located on the surface or in the core. The region contains 57.58% helix, 12.12% random coil, 24.24% extended strand, and 6.06% beta turn. We analyzed protein stability using I-mutant and Mupro servers. Due to R402 and E404K mutations protein stabilities were decreased (table2). All very high mutations are present in the helical regions of the REP (table2).

CONCLUSION

In this present work, using various bioinformatics platforms we made a comparison of the effects of mis-sense mutations in the REP region of parkin. The REP region is crucial for the E2 protein binding with parkin because it blocks the E2 binding site. Pathogenic mutation in this region may cause the structural modification in this region which subsequently affects its function. In this computational work we identified five most deleterious

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mutations which may be responsible for the onset of Parkinson disease. This is the first report on the analysis of mutations in the REP region of parkin. In order to analyze the effects of mutations, we used different software tools in order to get a comprehensive result. Further MD (molecular dynamics) simulation and some wet lab validation are required to understand the mechanism of the disease onset.

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Conflict of interest: The authors declare that there is no conflict of interest.

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Table1: All missense mutational prediction results are enlisted in the table.

Mutation	Region	SNPs&GO	Polyphen2	Proven	SNAP2	Aline GV&GD	Score
A379V	REP	Neutral	BENIGN	Neutral	Neutral	Class C65	1
V380I	REP	Neutral	BENIGN	Neutral	Neutral	Class C25	0
G385E	REP	Neutral	BENIGN	Neutral	Effect	Class C65	2
T387S	REP	Neutral	BENIGN	Neutral	Neutral	Class C55	1
Q389H	REP	Neutral	POSSIBLY DAMAGING	Neutral	Neutral	Class C15	1
R392G	REP	Neutral	BENIGN	Neutral	Effect	Class C65	2
V393I	REP	Neutral	POSSIBLY DAMAGING	Neutral	Neutral	Class C25	1
D394G	REP	Neutral	BENIGN	Deleterious	Effect	Class C65	3
D394N	REP	Neutral	POSSIBLY DAMAGING	Deleterious	Effect	Class C15	3
E395A	REP	Neutral	BENIGN	Neutral	Neutral	Class C65	1
R396G	REP	Neutral	BENIGN	Neutral	Neutral	Class C65	1
A397V	REP	Neutral	PROBABLY DAMAGING	Neutral	Neutral	Class C55	2
A398T	REP	Neutral	PROBABLY DAMAGING	Neutral	Neutral	Class C55	2
R402G	REP	Disease	PROBABLY DAMAGING	Deleterious	Effect	Class C65	5
E404K	REP	Neutral	PROBABLY DAMAGING	Deleterious	Effect	Class C55	4
S407P	REP	Disease	PROBABLY DAMAGING	Deleterious	Effect	Class C65	5
S407C	REP	Neutral	PROBABLY DAMAGING	Deleterious	Effect	Class C66	4
S407F	REP	Disease	PROBABLY DAMAGING	Deleterious	Effect	Class C67	5
K408N	REP	Neutral	PROBABLY DAMAGING	Neutral	Neutral	Class C68	2
E409K	REP	Neutral	PROBABLY DAMAGING	Neutral	Neutral	Class C55	2

Table2: Protein stability and secondary structure prediction results.

Mutation	I-mutant		MUpro server		SOPMA predicting secondary structure	Change of charge
	Stability	DDG value	Prediction stability	Confidence score		
R402G	Decrease	-1.87	DECREASE	-1.7740669	Helix	Positive>Neutral
E404K	Decrease	-0.25	DECREASE	-0.74384827	Helix	Negative>Positive
S407P	Increase	0.28	DECREASE	-1.6055901	Helix	Neutral>Neutral
S407C	Increase	0.21	DECREASE	-0.85479413	Helix	Neutral>Neutral
S407F	Increase	0.88	DECREASE	-0.68852927	Helix	Neutral>Neutral

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