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Molecular Insight Of Q126P PINK1 Mutation Parkinson's Disease

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Abstract

Innate Gene alterations affecting PARK2 and PARK6 are mostly responsible for Parkinson's disease. These genes encode the protein kinase PTEN-induced kinase 1 (PINK1) and the E3 ubiquitin ligase Parkin. Cooperation between Parkin and PINK1 regulates the mitophagy pathway, which recycles damaged mitochondria following oxidative stress. The E3 ubiquitin ligase Parkin belongs to the RBR family. Parkin protein's C terminal edge is home to the IBR domain. The IBR domain maintains the shape and adjustability of RING domains and displays zinc ion-dependent folding, havingtwo zinc-binding sites. Therefore, a parkin protein mutation in a zinc-binding site may result in inappropriate folding, which ultimately impairs the structure as well asfunction of the protein. Parkin that has been phosphorylated has worse autoinhibition because the phosphoserine group's altered surface electrostatics interfere with its intramolecular interaction. Early-onset Parkinsonism has been linked to homozygous PINK1 gene variants. A novel homozygous mutation (Q126P) in the PINK1 gene was reported in two German sisters diagnosed with aclinical presentation resembling Parkinsonism. The structural aspect of (Q126P) mutation was studied here. Furthermore, we show how a PINK1 mutation in the Parkinson protein family activates parkin, resulting in the expanded structure needed for E2-ubiquitin binding. All of these results emphasize the significance of the parkin activation process via the PINK1 phosphorylation signal and offer a structural view of the parkin and E3 ubiquitin ligase interaction method.

Keywords: Parkinson's disease, E2 ubiquitin, PTEN-induced kinase 1 (PINK1), RING domains, IBR domain, Mutation.

1.Introduction:

PD (Parkinson's disease) is among "the most common neurological situations. Dopaminergic neurons in the area of the substantia nigra of the midbrain" are lost in this condition, which results in its distinctive motor signs. About 5–10% of cases, most of which are sporadic and manifest later in life, are brought on by somatic genetic changes [1]. Among these,the majority of cases of early-onset recessive are brought on by mutations in PARK2 (Parkin) & PARK6 (PINK1). James Parkinson in his article "The Shaking Palsy," provided the earliest description of Parkinson's disease. About 1.5 to 2.0% of people over 65 who are elderly suffer from PD, which is an age-dependent disorder. The symptoms of PD include bradykinesia, stiffness, and tremors. In order "to sustain the integrity of the cellular proteins, damaged proteins are removed by the parkin

protein and E3 ubiquitin-ligase protein. Proteins that are going to be broken down are attached with ubiquitin molecules", that have been then broken down by an enzyme cascade mechanism which involves E2 conjugating enzyme, E1-activating enzyme, and E3 ligase. By using a shared route in order to stop selective autophagy of depolarized mitochondria, protein kinase PINK1 andparkin regulate mitochondrial quality [3, 4]. PD is mostly caused by genetic PARK2 and PARK6 gene abnormalities. "PINK1 is a protein kinase, and Parkin is an E3 ubiquitin ligase that these genes each encode. Parkin and PINK1 cooperation control the mitophagy process, which repairs damaged mitochondria following oxidative stress. The ubiquitin-like (UBL) domain of native parkin regulates its autoinhibited state, leading to inactivity. [5]. Parkin is a 52 kDa RING-between-RING (RBR) E3 ubiquitin ligase family member with 465 amino acid residues. The parkin protein has 4 zinc-coordinating RING" ("really exciting novel gene")-like domains, RING0, RING1, IBR, and RING2, in addition to a Ubl domain at both its N- and C- terminal ends. Cysteine and histidine residues in the parkin protein control 8 zinc (Zn) atoms overall. [6]. Parkin exhibits "E3 ligase activity in its greatly conserved RING-IBR-RING (RBR) domain architecture. The Ubl domain of parkin interacted with the C-terminal RING1 domain by generating a hydrophobic core around the protein's 44th residue. The C-terminal catalytic domain" and the RING0 domain are in communication. The interactions between the domains of the parkin protein are necessary for proper folding and function. 2 "zinc-binding sites exist in the conserved IBR domain" [7]. These zinc-binding sites are vital for proper binding interactions and for the parkin protein's proper folding. Parkin translocation and activation are directly impacted by the kinase activity of PINK1. First, "Ser65 in Parkin's Ubl domain is directly phosphorylated by Pink1. As a result, Parkin ligase activity rises. Second, ubiquitin is also phosphorylated on Ser65 by PINK1. Parkin interacts with phosphorylated ubiquitin" (pUb), which also boosts its activity. Additionally, in a feed-forward process, phosphorylated ubiquitin can function as a receptor for Parkin translocation to mitochondria [8].Therefore, ubiquitin and the Ubl must both be phosphorylated by PINK1 for Parkin to function in mitochondrial quality control, albeit there is debate regarding each protein's specific function. Through the ubiquitylation of particular mitochondrial proteins (VDAC1), Parkin makes it easier for the mitochondria to be cleared, which in turn attracts autophagic adapters (p62/SQSTM1) to carry out the final autophagy. Different PD-linked Parkin mutations can prevent the execution of mitophagy as well as the translocation to damaged mitochondria, exposing a multistep mechanism [9]. Previous research has demonstrated that Parkin and PINK1 share a similar genetic route, with PINK1 being more ambitious than Parkin. With those prior research, this PINK1/Parkin-directed route is contained. Additionally, we looked at the physical association capacities of PINK1 and The location and "functional effect of PD-related PINK1 mutations on the Parkin-dependent mitophagy and" Parkin mutants [10].

Materials and Methods

Model Building

PINK1 protein (UniProt id: Q9BXM7) andE3 ubiquitin ligase (UniProt id: O60260) protein are selected for building the model. Before downloading the Swiss Model model in pdb format, first download the Fasta format and convert the 126th positionof glutamine to proline(https://swissmodel.expasy.org) [11].

Preparation of Receptor

The human E3 ubiquitin ligase's 3D structure was obtained using RCSB-PDB (PDB ID: 5N38). It performs a receptor role.

Docking studies:

Using Patchdock, the molecular interactioninvestigation was carried out [12]. The docking procedure includes ligand and receptor preparation. The search criteria were chosen to bind with both mutant and wild-type proteins. We first choose the wild-type protein PINK1, which has 581 amino acids and a Uniprot ID of Q9BXM7, and we substitute proline forglutamine at position 126, creating amutant protein [17]. Then, these proteins, both wild-type and mutant, are docked with the 465 amino acid containing E3 ubiquitin ligase.

Protein -Protein complex prediction:

To forecast the affinities for protein-protein complexes following docking,select
these two docking results in the PRODIGY database as receptor and ligandand calculate
the binding affinities (ΔG) [13].

Discovery Studio: Visualize the wild-type and mutant docked complexes of these proteins on Discovery Studio to examine the intermolecular hydrogen bond between two amino acid residues.

2. Result and Discussion:

Instead of most other homozygous PINK1 mutations, the Q126P pathogenic mutation does not occur inside the kinase domain. Instead, the mutations at position glutamine residue Q126 affect the domain's highly conserved region (amino acids 55 to 155) that is shared by humans and the fish *Tetraodon nigroviridis*. A protein folding error is a likely result of switching from glutamine to proline in the amino acid chain [14]. Among 170 healthysamples treated as controls from the same population, this single-point mutation was not found. Furthermore, there is no evidence that a community-specific polymorphismcoincidentally segregating with the trait exists. The patient's clinical profile is typical for Parkinsonism caused by the PINK1 gene, with a benign course and satisfactory treatment [15]. It's possible that other mutations might have different effects and that the biochemical results are specific to the mutation that was found in our family. However this claim would be refuted by the very consistent clinical phenotype of PINK1-mutation carriers. Since mutations in either protein linked to Parkinson's disease (PD) obstructthis route, PINK1 and Parkin are necessaryfor the effective removal of contradictory mitochondria. Therefore, PINK1 mutations may prevent Parkin from being translocated in response to mitochondrial injury by altering the protein's stability and inducible subcellular localization. Notably,the presence of endogenous PINK1prevents PINK1 mutants from impairing Parkin translocation and mitophagic clearance. This supports the idea that PD- associated PINK1 mutations are inherited in a recessive manner [16].

Table 1. PRODIGY database results of two proteins

Protein-protein complex	ΔG (kcal mol^{-1})	K (M) at 25 °C
UBQ_WT	-28.7	8.3E-22
UBQ_MT	-25.9	1.0E-19

Due to their various connections to mitochondria, the three recessive Parkinsonism genes—Parkin, DJ-1, and PINK1—may have the potential to provide protection against oxidative stress or mitochondrial dysfunction. Recent research in Drosophila demonstrates that over-expressing PARKIN can reverse the mitochondrial dysfunction caused by PINK1 deletion. According to this, PARKIN and PINK1 act, at least in part, in the same pathway, with PINK1 functioning upstream of PARKIN. According to a recent in vitro study, the proteins PINK1 and DJ-1 substantially connect in the cell and work together toshield it from oxidative stress brought on by MPP+. According to other cell culture studies, wild-type PINK1 shields neurons from stress-induced mitochondrial dysfunction and death. The PINK1 mutations reverse this benefit, while the exact mechanism is unknown. Following docking, choose these two results as the receptor and ligand in the PRODIGY database to forecast the binding affinities in protein-protein complexes and protein-small ligand complexes (Table 1) [17].
On the other hand, examine the intermolecular hydrogen bond betweentwo amino acid residues by seeing this protein's wild type and mutant forms on Discovery Studio (Table 2).

Table 2. Intermolecular hydrogen bondsof wild-type protein and mutant protein.

ProteinName	Chain Description	Complexnames	Hydrogen Bond
PINK1 (Protein kinase PTEN induced kinase 1)	A Chain (Receptor_ E3 Ubiquitin)	UBQ_WT	A:LYS151:NZ-B:GLU154:OE1 A:ARG305:NH2- BGLU154:OE1 B: ARG152:NH2- A: THR222:OG1 B:GLU206:N-A:PRO345:O B:GLN327:NE2-A:GLU353:O A:ASN313:ND2-B:VAL285:O AGLN317:NE2-B:LEU288:O A: ASN356:ND2- B: ASP:366:OD1 B: GLY204:CA-AlLCYS347:O B:SER284:CB-A:GLU309:OE1 B:GLY290:CA-A:CYS337:O A:CYS337:CA-B:ASP294:OD1

	B Chain (Ligand_ PINK1)		A:ARG366:CD-B:GLU203:O A:GLY375:CA-B:THR198:0
		UBQ_MT	BARG68:NH1-AASP243:OD1 B:ARG71:NH2-A:GLU49:OE1 A:GLN34:NE2-B:SER123:O A:ARG156:NH2-B:GLY105:O A:THR237:OG1-A:CLZ502:CL A:ARG396:NH1-A:THR242:O B:ARG68:NH2-ATHR242:O B:ARG80:NE-A:SER246:O B:GLN134:NE2-A:GLY12:O

Conclusion

Our analysis "to predict the binding affinity of protein–protein complexes" between a wild-type protein and mutant protein showeda higher binding affinity with wild-type PINK1 with E3 Ubiquitin ligase.

Conflict of interest

None

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