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RESEARCH ARTICLE

Computational Analysis of Parkinson's disease Associated Pink1 Gene:A Neuroinformatics Approach.

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Abstract

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's, caused primarily by the loss of dopaminergic neurons of the Substantia-Nigra. Mutations in the putative-induced kinase - a mitochondrial serine/threonine-protein kinase encoded by the PINK1 gene have been found in families with recessive early-onset Parkinson's disease.

Neuroinformatics allow researchers to quantitatively confirm theories by computational models. In genomics, the effectiveness of available databases and the application of theoretical and computational models demonstrate solution of complex problems.

The present study is based on computational analysis, to explore in-depth knowledge about Parkinson's disease associated PINK1 gene, using advanced bioinformatics tools such as, STRING database, KEGG pathway, NEXT PROT, BioGrid database to find the protein-protein interactions, their metabolic pathway and protein sequence.

Neuroinformatics approach in the current study expressed unique possibilities to enhance understanding towards the biological function of the particular gene like PINK1 gene which is associated with the progression of Parkinson's disease.

INTRODUCTION

The molecular basis of genetic diseases is possible with the advancements in genomic technologies that strongly boost up research studies on genetic associations with the disease. The effectiveness of protein-protein interactions in determining gene–disease associations, identify a set of candidate genes, either related to the disease or may have physical interaction with the gene product (1). The usually proposed computational approaches reveals two sources of information to predict associated genes with diseases are linkage intervals and physical interaction networks. Verity of different techniques uncovers the associations between gene and disease taking an integrative approach such as; genes expression, biological pathways, protein sequences, and numerous phenotypic traits of diseases.

PD is the second most common neurodegenerative disorder in the Western world and presents as a progressive movement disorder. The pathological features of PD characterized by loss of dopaminergic neurons in the region of substantia nigra and neuronal intracellular Lewy body inclusions and clinically characterized by tremor, rigidity, postural instability and bradykinesia (2).

Molecular mechanism of the PD has been significantly understood by the identification of other familial forms and cloning of casual genetic mutations. The spotting of alteration in genes that is involved in encoding of α-synuclein, Parkin, DJ-1 and PINK1 has became the platform for the progression of molecular mechanisms associated with this disease (3-5).

PD has been discriminated by the mitochondrial dysfunction, oxidative stress and inflammation based on multiple genetic factors and other influences of environmental factors, leading the dopaminergic neurons to apoptosis. Six causal genes for Mendelian inherited PD have been identified to date, markedly relevant to the ubiquitin-proteasome pathway in the molecular pathogenesis of dopaminergic cell death. Recent studies have also determined the entanglement of genetic factors in the pathogenesis of sporadic PD (**6-8**).

Exploration for the pathogenesis of this disease has been triggered in recent years by the pinpoint of several genes susceptive for familial forms of parkinsonism (9) Triplication and missense mutations in the α-synuclein gene cause autosomal-dominant (AD) familial parkinsonism, whereas loss-of-function mutations (large deletions and truncations) in the Parkin, DJ-1, and PINK1 genes cause autosomal-recessive (AR) inheritance of Parkinsonism syndromes (10). The diagnosis of PD is made only on the basis of clinical criteria and No biological marker has been identified yet that strongly confirms the diagnosis (11).

PD and related neurodegenerative disorders thriving concerns of the health care system. In greater number of cases, the cause of the disease is still unperceived, and its elucidation being among the major challenges of the neurosciences. Combination of genetic, pathologic and molecular findings provide increasing evidence that the pathways identified through the cloning of different disease genes are interacting on different levels and share several major pathogenic mechanisms (12-13)**.**

The present study is designed to explore the affiliation of PINK1 gene with Parkinson's disease by using bioinformatics analysis. The prior bioinformatics tools such as, KEGG pathway, STRING, NEXT PROT, HPRD, RCSP and Bio-GRID databases were used to find the protein-protein interactions, their metabolic pathway and protein sequence of the PINK1 gene.

MATERIALS AND METHODS

Computational analysis made possible in-depth knowledge of PINK1 gene which is associated in the progression of PD by using bioinformatics tools like Gene Bank, STRING's, KEGG Pathway, Uniprot /Swissprot, NEXT PROT, HPRD, RCSP and BioGRID databases.

The KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway database records networks of molecular interactions in the cells, and variants specific to particular organisms (http://www.genome.jp/kegg/). The Reactome pathway database (http://www.reactome.org/); Uniprot/ Swissprot database that provides the protein knowledgebase includes complete and reference proteome sets. STRING 8.3 (http://string-db.org/) was used to explore the biological associations of knowledge base differentially expressed protein.

RESULTS

The present study represents the bioinformatics analysis of PINK1 gene which is mutated in some forms of PD. Table#1 depicted the basic information about Pink1 which was available on gene bank & gene card suggested that it is a protein coding gene having a protein name "putative induce kinase1" it is refer to a family of serine/threonine kinase 1 predicted for its various phosphorylation behavior. The molecular weight of PINK1 gene is 62,769 Da. Due to defects in functions of PINK1 gene leads the down level of dopaminergic neuron which is the leading factor in the progression of PD.

The chromosomal location of pink 1 gene has been actuated by the gene card figure out that it is present on chromosome 1 at position 36.12 Figure 1 and sequence on the gene of interest depicted in Figure 2.

The KEGG metabolic pathway of PINK1 gene substantiate that PD is a progressive neurodegenerative movement disorder that results primarily from the death of dopaminergic (DA) neurons in the substantia nigra (SN). Mutations in alpha-synuclein, UCHL1 (an ubiquitin carboxy-terminal hydrolase L1), Parkin, DJ1 (a Parkin-associated protein involved with oxidative stress), and PINK1 are known to cause early-onset PD (Figure 3).

The STRING network predicts the functional association of pink1 gene with PARK2, HTRA2, TRAP1, CDC37, ATP13A2, RICTOR, DUSP6, and ODC1. The boundaries represent the predicted functional associations. These boundaries may be drawn with up to 7 differently colored lines - these lines represent the existence of the seven types of evidence used in predicting the associations. A red line indicates the presence of fusion evidence of PARK2, HTRA2, PARK7 with PINK1 a green line - neighborhood evidence and a blue line -co occurrence evidence with TRAP2, DUSP6, ODC37, ATP13A2, HTRA2, PARK2, PARK7, FABP4, RICTOR, a purple line indicates experimental evidence with TRAP1& CDC37, a yellow line with FBP4 having text mining evidence; a light blue line - database evidence associated with ATP13A2, PARK2, PARK7 a black line - co expression evidence predicted between TRAP1 & CDC37 (Figure 4).

Furthermore**,** BioGRID database helps in determination of Pink1 partners under the influence of environmental and genetic factors UBC, PAG2, CDC37, CRLS1, EED, HSP90AA1, PARK2, PARK7, and SRM1. Physical factors include Affinity Capture-Luminescence, Affinity Capture-MS. Affinity Capture-RNA, Affinity Capture-Western, Biochemical Activity, Co-crystal Structure, Co-fractionation, Co-localization and Co-purification. Genetic factors include Dosage Growth Defect, Dosage Lethality (Figure 5).

UniProt provide the accessible resource of protein sequence and functional information. The Uniprot-Knowledgebase is the central access point for extensive curate protein information, including function, classification, and cross-reference. Additionally, NeXtProt that provides an original way of visualizing proteins entries: they can be seen from three different perspectives such as, the 'Protein', the underlying 'Gene' and the 'References' used to annotate it.

TABLE#1 CLASSIFICATION AND FUNCTION OF PINK 1 GENE

GENE	PINK1
Organism	Homosapiens
Gene type	Protein coding
Family name	Protein kinase » Ser/Thr protein kinase entry whose protein existence is based on evidence at protein level
Protein	Serine/threonine-protein kinase PINK1, Mitochondrial protein.
Alt. Names/Synonyms	BRPK; FLJ27236; PARK6; PINK1; protein kinase BRPK; PTEN induced putative kinase 1; Serine/threonine-protein kinase PINK1.
Locus	1p36.12
Molecular Weight	62,769 Da
Basal Isoelectric Point	9.43 Predict pI for various phosphorylation states
Tissue specificity	Highly expressed in heart, skeletal muscle and testis, and at lower levels in brain, placenta, liver, kidney, pancreas, prostate, ovary and small intestine. Present in the embryonic testis from an early stage of development.
Cellular Component	Mitochondrial outer and inner membrane cytoskeleton; cytosol; nucleus; chromatin.
Molecular function	1. Protein serine/threonine kinase activity; 2. Protein binding; 3. Ubiquitin protein ligase binding; 4. Magnesium ion binding; 5. Kinase activity; /ATP binding.
Biological process	1. Cell death; 2. Positive regulation of dopamine secretion, peptidyl-serine phosphorylation. 3. Positive regulation of synaptic transmission, 4. Mitochondrion degradation; response to stress, regulation of protein complex assembly. 5. Negative regulation of neuron apoptosis, protein amino acid phosphorylation.
Catalytic activity	ATP+ a protein = ADP+ a phosphoprotein
Cofactor	Magnesium

FIGURE-1: Chromosomal Location of PINK1 Gene.

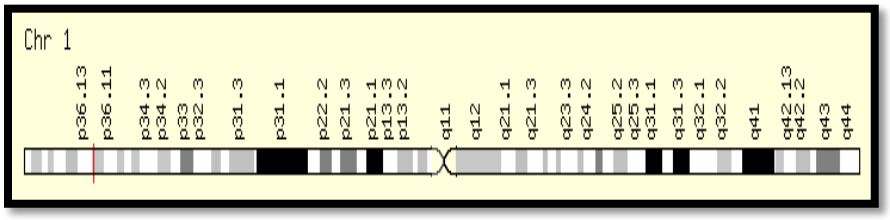


FIGURE-2: Protein Sequence of PINK1 Gene by FASTA Format.

PINK1_HUMAN Serine/threonine-protein kinase PINK1, Homo sapiens

MAVRQALGRGLQLGRALLLRFTGKPGRAYGLGRPGPAAGCVRGERPGWAAGPGAEPRRVGLGL
PNRLRFFRQSVAGLAARLQRQFVVRAWGCAGPCGRAVFLAFGLGLGLIEEKQAESRRAVSACQEI
QAIFTQKSKPGPDPLDTRRLQGFRLEEYLIGQSIGKGCSAAVYEATMPTLPQNLEVTKSTGLLPGRG
PGTSAPGEGQERAPGAPAFPLAIKMMWNISAGSSSEAILNTMSQELVPASRVALAGEYGAVTYRK
SKRGPKQLAPHPNIIRVLRAFTSSVPLLPGALVDYPDVLPSRLHPEGLGHGRTLFLVMKNYPCTLR
QYLCVNTPSPRLAAMMLLQLLEGVDHLVQQGIAHRDLKSDNILVELDPDGCPWLVIADFGCCLAD
ESIGLQLPFSSWYVDRGGNGCLMAPEVSTARPGPRAVIDYSKADAWAVGAIAYEIFGLVNPFYGQ
GKAHLESRSYQEAQLPALPESVPPDVRQLVRALLQREASKRPSARVAANVLHLSLWGEHILALKN
LKLDKMVGWLLQQSAATLLANRLTEKCCVETKMKMLFLANLECETLCQAALLLCSWRAAL

FIGURE-3: Metabolic Pathway of PINK1 Gene

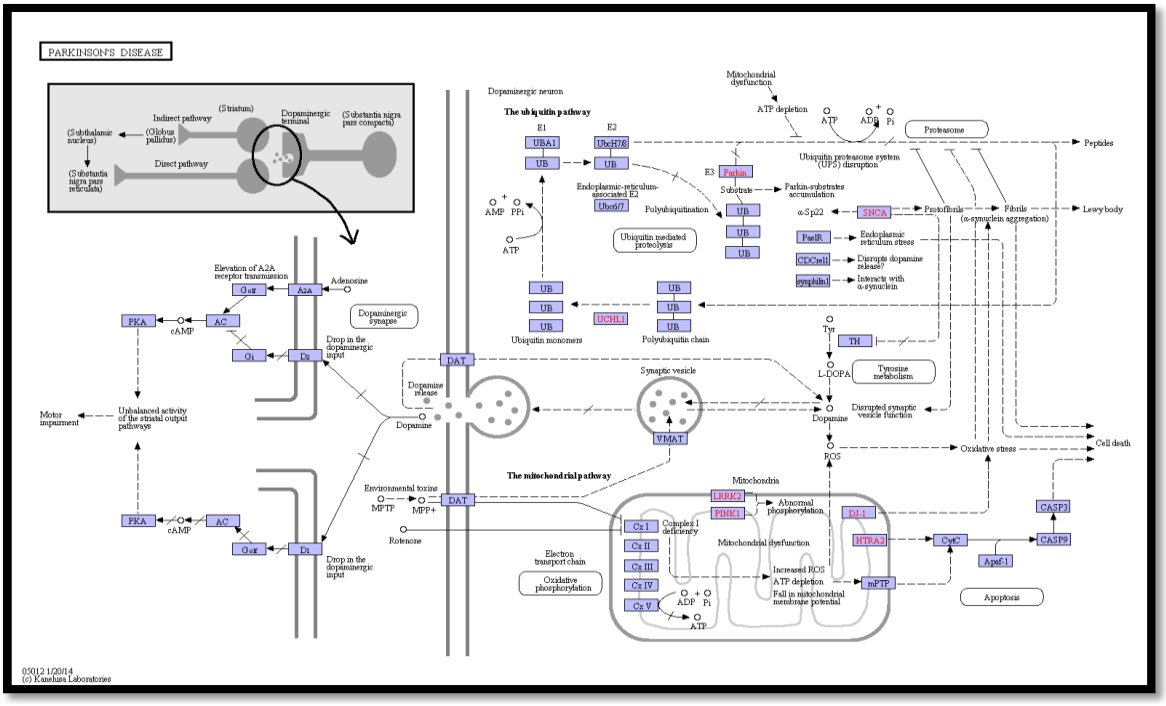


FIGURE-4: String Network Predict Functional Partners of Pink 1 Gene.

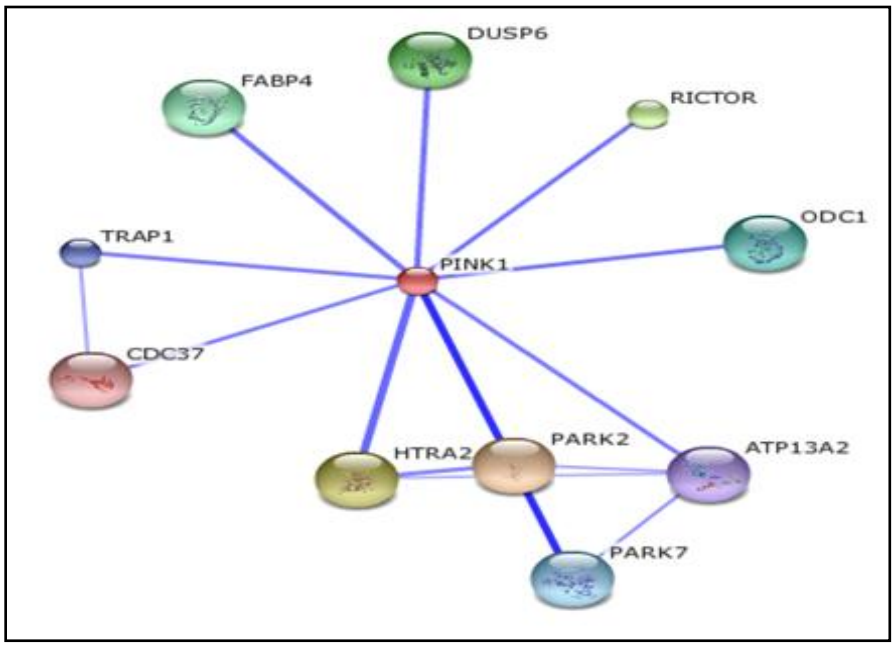
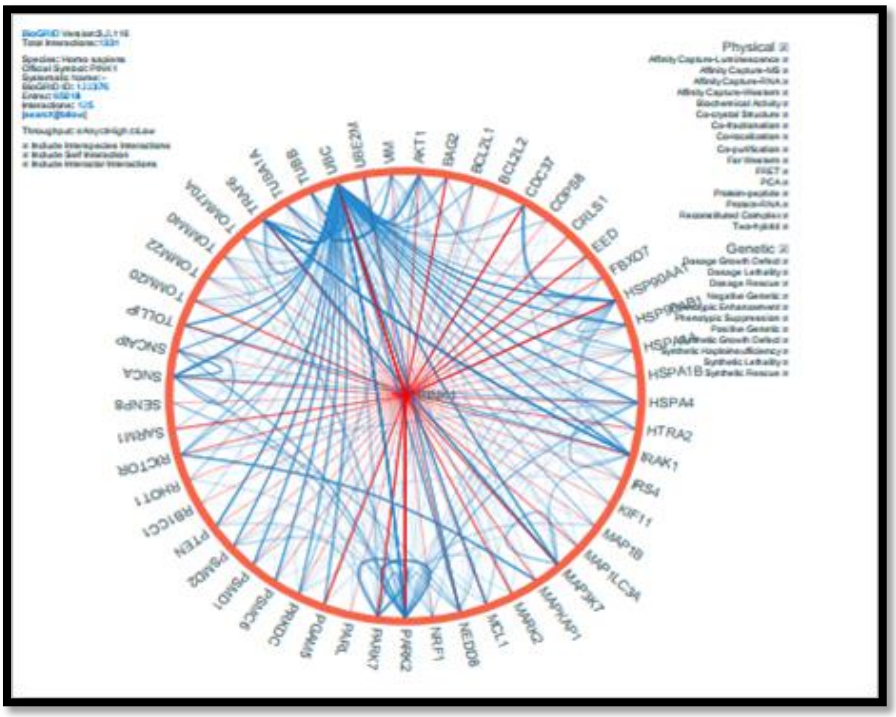


FIGURE-5 PINK1 Interaction with Different Proteins by BioGrid .



DISCUSSION

Computational analysis aids in the prediction of individual gene function, cellular interaction networks and their involvement in regulation of cellular physiology. With the increase in availability of human protein interaction data, the focus of bioinformatics development has shifted from understanding networks encoded by model species to understanding the networks underlying human disease (14). By using biocomputing techniques we extract out the informative data about PINK1 gene. Genomic location, function, catalytic activity, sub cellular location, Iso-electric point and family belonging of PINK gene have been accumulated by gene bank/gene card data analysis.

The present study comprises on the computational bioinformatics analysis of PINK1 gene or the protein Parkin, which is mutated in some forms of familial Parkinson's disease. The frequency of PINK1 mutations is in the range of 1%–9%, with considerable variation across different ethnic groups.

PINK1 gene - the Putative-Induced Kinase - mitochondrial Serine/Threonine-Protein Kinase - a 581 amino acid ubiquitously expressed protein kinase. It consists of an amino-terminal 34 amino acid mitochondrial targeting motif, a conserved serine–threonine kinase domain (amino acids 156–509; exons 2–8), and a carboxy-terminal autoregulatory domain. Two-thirds of the reported mutations in PINK1 are loss-of-function mutations affecting the kinase domain, demonstrating the importance of PINK1's enzymatic activity in the pathogenesis of PD.

A variety of mutations in the PINK1 and Parkin genes cause early-onset PD in humans. PINK1 might accumulate in damaged mitochondria because of increased synthesis and/or reduced degradation of the protein. Excessive PINK1 accumulation on mitochondrial membranes is therefore sufficient to recruit Parkin and activate mitochondrial autophagy, even in the absence of membrane depolarization.

KEGG Pathway mapping is the process to map molecular dataset. KEGG describes metabolic pathway of PINK1 gene which detect the defective gene involved in disease. In early-onset PD various mutations are known to be involved like mutation in alpha-synuclein, a ubiquitin carboxy-terminal hydrolase L1 (UCHL1), Parkin, DJ1 and PINK1 etc., contributes to the damage and subsequent loss of DA neurons through common mechanisms results in proteasome dysfunction, mitochondrial impairment and oxidative stress.

PINK1 and PARKIN have been identified as the causal genes responsible for hereditary recessive early-onset Parkinsonism. The loss of dopamine neurons in the striatum could results in reduced activation of the direct pathway and in a disinhibition of the indirect pathway, which is associated with the elevation of adenosine A2A receptor transmission. Such unbalanced activity of the striatal output pathway is at the basis of the motor impairment observed in PD.

The String's data base quantitatively integrate interactions data i.e. interaction of proteins with different proteins it shows the strong interactive proteins also determine the co expression in another organisms pathway data set. String network predict the functional partners of pink1 gene PARK2, HTRA2, TRAP1, CDC37, ATP13A2, RICTOR, DUSP6, ODC1 (Figure-2). The predicted genes tasks are given below: PARK2 - Parkinson disease 2, parkin; a multiprotein E3 ubiquitin ligase complex that plays a role in the removal and/or detoxification of abnormally folded or damaged protein via ubiquitin proteasome pathway. Loss of this ubiquitin ligase activity appears to be the mechanism underlying pathogenesis of PARK2. HTRA2- serine peptidase 2., illustrate proteolytic activity against a non-specific substrate, beta-casein leading to an augment in caspase activity to promote or induce cell death either by direct binding to and inhibition of BIRC proteins - a inhibitor of apoptosis proteins, IAPs. Dual specificity phosphatase 6 (DUSP6)-; Inactivates MAP kinases has a specificity for the ERK fatty acid binding protein 4 involve in lipid transport in adipocytes, binds with both long chain fatty acids and retinoic acid and delivers to their cognate receptors in the nucleus. TNF receptor-associated protein 1 (TRAP1) - an important chaperone that expresses an ATPase activity. CDC37- microRNA 1181; Co-chaperone that binds to numerous kinases and promotes their interaction with the Hsp90 complexresulting in stabilization and promotion of their activity. PARK7- a sensor for oxidative stress. It functions as a redox-sensitive chaperone to prevent aggregation of SNCA in order to protect neurons from oxidative stress that results in cell death. RICTOR is the independent companion of MTOR that regulates the process of cell growth and survival via phosphorylation in response to hormonal signals.

Recent studies provided confirmation that in elimination of damaged mitochondria from the mitochondrial network, PINK1 and Parkin plays a significant role by using general single pathway. Mitochondria are stabilized via lowering in membrane potential through PINK1, further helps to recruits Parkin from the cytosol to mitochondria, leading its enzymatic activation towards mitophagy (15). This mitochondrial damage may increase susceptibility in the Substantia Nigra neurons due to greater oxidative stress and/or because inability of neurons to regenerate as occurs in other cell types.

An increased risk of developing PD has been associated with variants in several PARK-designated (SNCA, UCHL1, LRRK2, PARK 16, GAK) and a few other genes (MAPT, GBA, NAT2, INOS2A, GAK, HLA-DRA, and APOE*)*. Such risk factors polymorphisms/mutations were mostly identified based on GWAS and functional candidate approaches. As the identification of a PD gene mutation—especially in a patient with early-onset PD frequently has an important impact that goes beyond a reduction in diagnostic uncertainty. Indeed, in cases of PD the identification of specific mutations can provide information on prognosis and will have an effect on treatment choices that were initially suspected to be psychogenic.

The BioGRID - Biological General Repository for Interaction Datasets provides interaction between the biological and computational datasets. The BIOGRID associated Pink1 partners includes UBC, PAG2, CDC37, CRLS1, EED, HSP90AA1, PARK2, PARK7, and SRM1, under epigenetic influence.

CONCLUSION

Neuroinformatics approach in the current study expressed unique possibilities to enhance understanding towards the biological function of the particular gene like PINK1 gene which is associated with the progression of Parkinson's disease. The current accessibility of molecular interaction networks in humans has revolutionized the importance not only of the proteins themselves, but of their inter-relationships. As Human network data remains elusive may be due to the biological, technological and/or algorithmic challenges still faced regarding molecular networks of a particular disease. However, utilizing advancement in these networks updates our analysis about disease progression, diagnosis, and treatment.

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