A NOVEL MISSENSE MUTATION (c.1982A>C) IN *FCHSD1* CAUSES AUTOSOMAL RECESSIVE EARLY ONSET PARKINSON'S DISEASE IN A CONSANGUINEOUS PAKISTANI FAMILY

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Abstract
Parkinson's Disease (PD) is a neurodegenerative disorder characterized mainly by degenerative loss of dopamine producing neurons and accumulation of alpha synuclein in substantia nigra pars compacta (SNpc) of midbrain. Impairment of autophagy is believed to be common among multiple neurodegenerative disorders. The disruption in autophagic process and improper removal of accumulated proteins are linked with various neurodegenerative disorders including Parkinson's disease. Whole Exome Sequencing (WES) was done on extracted DNA of the patient having PD to identify novel causative variants for underlying disorder. Identified variant was then validated in other family members through Sanger's sequencing. A novel missense variant NM_033449.3:c.1982A>C (p.Asp661Ala) in *FCHSD1* gene was identified through WES. Segregation of the mutation in other family members was confirmed through Sanger's sequencing. The identified mutation (c.1982A>C) in *FCHSD1* gene is responsible for autosomal recessive Parkinson's disease in a consanguineous Pakistani family.

Background: Parkinson's Disease (PD) is a neurodegenerative disorder characterized mainly by degenerative loss of dopamine producing neurons and accumulation of alpha synuclein in substantia nigra pars compacta (SNpc) of midbrain. Impairment of autophagy is believed to be common among multiple neurodegenerative disorders. The disruption in autophagic process and improper removal of accumulated proteins are linked with various neurodegenerative disorders including Parkinson's disease.

Aims: The aim of this study was to describe the genetic variants responsible for Parkinson's Diseases in a consanguineous family.

Objective: To identify novel causative mutation responsible for autosomal recessive Parkinson's disease in a consanguineous Pakistani family.

Methods: Whole Exome Sequencing was done on extracted DNA to identify novel causative

variants for underlying disorder. Identified variant was then validated in other family members through Sanger's sequencing.

Results: A novel missense variant NM_033449.3;c.1982A>C in FCHSD1 gene was identified through Whole Exome Sequencing (WES). Segregation of the mutation was confirmed through Sanger's sequencing.

Conclusion: Identified mutation (c.1982A>C) in FCHSD1 gene is responsible for autosomal recessive Parkinson's disease in a consanguineous Pakistani family.

Keywords: Parkinson's disease, *FCHSD1,* mTOR, Whole Exome Sequencing, Autosomal Recessive, Early Onset Parkinson disease

INTRODUCTION

Parkinson's Disease (PD) is a progressive neurodegenerative disorder. The main characteristic features of PD is degeneration of dopaminergic neurons in substantia nigra pars compacta (SNpc) of midbrain along with accumulation of Lewy bodies in neurons [1]. With the progression of this neuronal loss, typical motor symptoms such as rigidity, bradykinesia, postural instability and tremors becomes evident [2]. Although complete pathogenesis of PD is not fully understood but the literature linked it with toxic and abnormal regulation of alpha synuclein, oxidative stress and mitochondrial dysfunction [3]. In addition to this, PD development is believed to involve complex interactions between environment and genetic predispositions. Genetic mutations in the genes associated with PD account for 5 to 10 percent of all PD cases while majority of PD cases are classified as idiopathic PD (IPD) [4].

FCH and double SH3 domain 1 *(FCHSD1)* gene, located on the chromosome 5q31.3, is a member of group of genes implicated in protein synthesis through mechanistic target of rapamycin (mTOR) kinase signaling pathway [5]. *FCHSD1* is thought to play a role in actin dynamics. Mutations in *FCHSD1* gene are related with the PD pathogenesis through mTOR pathway [6]. Exact mechanism of how mTOR pathway impacts PD pathogenesis is not fully understood, but evidences from studies highlight its significant role in survival, growth, cell proliferation, metabolism and death [5]. Specific genetic loci of *FCHSD1* along with *SNCA*, *GSK3B* and *SKT11* have already been associated with elevated risk of PD in Spanish Population [7].

mTOR is a serine/threonine protein kinase that belongs to phosphoinositide-3-kinase (PI3K)-related kinase family [8]. It consists of catalytic component of two different multiprotein complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [9]. When mTOR is associated with Raptor, then it is termed as mTORC1, which governs cellular events like translation and autophagy [10] and when associated with Rictor, it is named as mTORC2 and governs Akt trophic activity and actin polymerization, which controls cellular processes like survival and growth [11]. In physiological condition, this pathway controls fundamental processes inside brain like neuron survival, memory formation, synaptic plasticity and neural development [12]. A study proposes the potential link between abnormal mTOR pathway and PD [13]. Dysregulation of PI3K/AKT/mTOR pathway is frequently observed in brains of patients suffering from Alzheimer's disease (AD) and Parkinson's disease (PD) [14]. Impaired mTOR and Akt are activities is observed in substantia nigra of brain of postmortem PD patients [15]. Elevated expression of wild type mTOR offers protection against neuronal damage [16].

mTOR is an important regulator of autophagy [17]. Animal models of PD and AD show altered autophagy associated with dysregulation of PI3K/AKT/mTOR pathway [18]. Increased synthesis of protein and decrease in autophagy in PD is associated to alpha synuclein aggregation which leads to degeneration of dopaminergic neurons. So, abnormal mTOR pathway can lead towards PD pathology in brain [19]. Accumulation of α-synuclein is observed in both familial and sporadic PD [20]. Analysis of temporal cortex from PD patients having α-synuclein accumulation shows enhanced levels of mTOR protein expression [21]. Moreover, overexpression of α-synuclein, has ability to inhibit autophagy by stimulating mTOR activity and replicate PD symptoms. While on the other hand, rapamycin, mTOR inhibitor, can restore the stimulated mTOR activity induced by

overexpression of α-synuclein [22]. Inhibition of mTOR pathway by rapamycin or its analogs,

rapalogs, is effective in prevention of dopaminergic neurons loss in animal models of PD [23]. While it is also observed in few studies that activation of the PI3K/AKT/mTOR pathway typically promotes neuronal survival and protection while inhibiting autophagy via mTOR activation [24]. RTP801/REDD1, a stress-associated protein, encoded by DDIT4 exhibits overexpression in neurons of substantia nigra pars compacta (SNpc) in PD patients as compared to controls [25]. RTP801 interacts with inhibitor of mTOR pathway i.e. TSC2 and results in neuronal death. This can be a possible mechanism that could cause SNpc neuron loss in PD. Overexpression of RTP801 is also observed in cellular PD models (6-OHDA, MPP+, or rotenone) and PD animal models along with a decrease in mTOR activity in both cases [26]. *FCHSD1* gene mutation shows significant association with expression of DDIT4, which encodes RTP801 [27]. RTP801, in turn, negatively regulates mTOR and Akt. As already known, RTP801 is upregulated in PD models and in PD human brain [25]. Numerous studies have been conducted to find a possible signaling pathway resulting in PD development. However, a proper connection between *FCHSD1* gene and PD is lacking. These findings suggest a potential interaction between genetic variations in the *FCHSD1* gene and the modulation of the mTOR pathway.

Here we studied a consanguineous family with PD phenotype from South Punjab and identified a novel missense variant in a known PD gene.

In accordance to the action mechanism proposed for PD induced by mTOR pathway, we hypothesize that genetic mutations which modify mTOR pathway can cause susceptibility to PD.

Materials and Methods
Enrollment of family
A family PKIUPD016 having six individuals affected with PD was recruited from Southern Punjab region of Pakistan as shown in Fig (1). The family was enrolled in the current study on the basis of inclusion criteria for our study i.e. number of affected individuals, clinical diagnosis, consanguinity and pattern of inheritance. The family was diagnosed with PD by a Neurophysician in accordance with the criteria of United Kingdom Parkinson's Disease Society (UPDRS) and Hoehn and Yahr criteria. Patients having PD also fulfilled UK Brain Bank Criteria for PD diagnosis. The family was classified as familial as more than three members were affected with the similar phenotype. The family history was assessed. A total of 5 individuals; two affected and three unaffected individuals including siblings and their children were included in this study. Written consent was obtained from all participating family members or their legal guardians in the study for recording their clinical history and genetic testing. This study was approved by the Human Ethics committee and the Institutional Bio-safety committee of The Islamia University of Bahawalpur, Pakistan.

Clinical Examination
All available members of the family were examined physically, neurologically, and psychiatrically. UPDRS questionnaire was used to obtain detailed clinical history of the patients. The detailed clinical features of patients having PD are given in Table 1. All the studied affected individuals were suffering from cardinal features of PD. However, control healthy individuals enrolled in this study had no sign and symptoms of PD or any other related disorder. The family members participating in the study refused to undergo MRI scanning.

Blood Sample Collection
10 ml blood sample was taken by venipuncture from affected and healthy family members in EDTA containing falcon tubes. Genomic DNA was extracted from blood samples by method described by Chacon-Cortes and Griffiths, 2014 [28]. DNA quantity and quality were assessed by spectrophotometry and gel electrophoresis, respectively.

Whole Exome Sequencing and Segregation Analysis
Whole exome sequencing (WES) was done on DNA samples of individuals IV:2 and IV:3 in order

to identify causative variants by use of Twist Comprehensive Exome Panel (Twist Biosciences) and after that sequenced by using HiSeq 4000 instrument (Illumina). Sequencing achieved an average coverage ranging from 100-200X at every nucleotide position. Raw reads were aligned to human genome reference sequence (build hg19) by use of Bovoalign software. Base quality score recalibration was conducted using HaplotypeCaller (GATK, v.4.0.3.0). Duplicates were eliminated using Picard (v.2.14.0-SNAPSHOT). Variants including SNVs and small insertion/deletion (Small InDels) were identified by use of GATK (GATK v4.0). Inclusion criteria was adapted to filter out variants with low allele frequency, deleterious effects, intolerant and pathogenic variants i.e. gnomAD < 0.01, Polyphen2 score > 0.5, SIFT score < 0.05, Mutation Taster score > 0.5 respectively and CADD > 20. GERP++ score was adjusted to be above 3 in order to maintain conservation.

Segregation analysis

Segregation analysis was conducted through Sanger's sequencing to validate same variants in other affected and healthy members of family. Segregation analysis was done by designing primers for targeted region flanking 319 bp up and down of targeted region. Primers were designed using Primer 3 Plus in silico tool. Specificity of primers was checked using In silico PCR from UCSC Genome Browser. GC contents were adjusted between 50-55% and Tm was set between 59.2-60.0^{0}C. DreamTaq Green PCR Master Mix (2X) (K1081) was used for PCR amplification. Amplified products were then cleaned using ExoSAP-IT™ (Express PCR Product Cleanup Reagent Catalog number: 75001.200.UL). Amplified products were run on the 1.2% agarose gel prior to send for sequencing, which was then done commercially. Chromatograms of Sanger's sequencing were then analyzed using Chromas version 2.6.6.

Results

WES unveiled a novel homozygous missense variant NM_033449.3:c.1982A>C (p.Asp661Ala) in exon 19 of *FCHSD1* gene in the proband of this family. The presence of this variant was confirmed in all available affected and healthy individuals through Sanger's sequencing as shown in Fig (2). This variant did not appear in Varsome and Genome Aggregation Database. Three in silico bioinformatics tools i.e. PolyPhen2, SIFT and Mutation Taster predicted this variant as damaging and CADD score was 23.2. Both of the affected individuals (IV:2 and IV:3) possess this variant in homozygous form and in available healthy individuals (V:1, IV:6 and IV:5) carried it in heterozygous form.

Table 1 Clinical Features of patients carrying *FCHSD1* mutations

Clinical Feature	IV:2	IV:3	V:1	IV:6	IV:5
Family ID	PKIUPD016	PKIUPD016	PKIUPD016	PKIUPD016	PKIUPD016
Age at Evaluation	65	55	40	58	43
Age of Onset	20	20	-	-	-
Duration	45	35	-	-	-
Mutational Status	Homozygous	Homozygous	Heterozygous	Heterozygous	Heterozygous
Sex	Female	Male	Male	Female	Male
Progression of disease	++	+	-	-	-
Gait Ataxia	+	+	-	-	-
First Symptom	Rest Tremor	Rest Tremor	-	-	-
Clinical Status	Affected	Affected	-	-	-
Clinical Features	RT, B, PI, R	RT, B, PI	-	-	-
Asymmetry	Bilateral	Bilateral	-	-	-
Dysarthria	++	++	-	-	-
Dysphagia	+++	++	-	-	-
UPDRS Part III score (On/Off Medication)	NA/28	NA/21	-	-	-
Hoehn and Yahr Scale	Stage 3	Stage 2	-	-	-
Hoffman Sign	Positive	Positive	-	-	-

Schwab and England Scale	80	80	-	-	-
Mental Retardation	N/A	N/A	-	-	-
Rest Tremor	Present	Present	-	-	-
Diadochokinesia	Abnormal	Abnormal	-	-	-
Postural Instability	++	++	-	-	-
Drooling	+++	++	-	-	-
Depression	N/A	+	-	-	-
Dementia	-	-	-	-	-
Dystonia	-	-	-	-	-

+mild, ++moderate, +++severe
RT: Rest Tremor, B: Bradykinesia, PI: Postural Instability, R: Rigidity
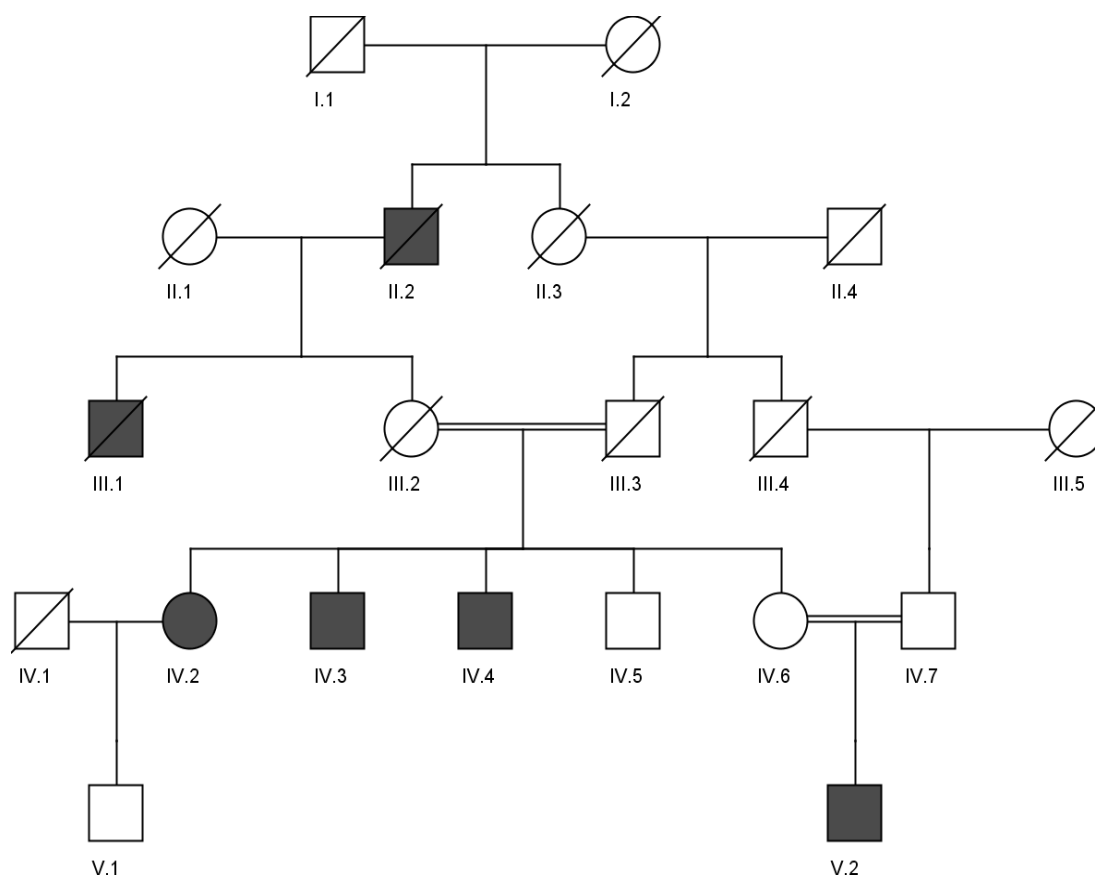

Fig 1. Pedigree of PKIUPD016 harboring novel missense mutation. Each generation is specified by the Roman numeral and individuals in the particular generation by Arabic numeral. Circles refers to females, squares to males, filled signs to affected individuals and slashed lines to the deceased persons.
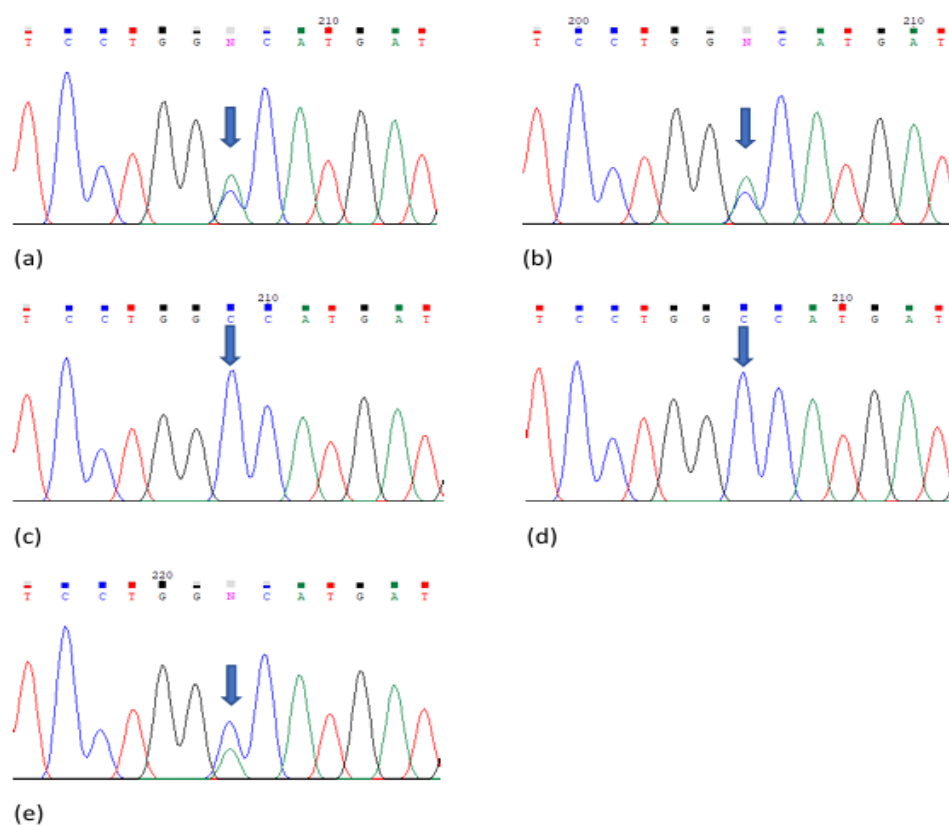
Fig 2. Shows results of Sanger's sequencing. Arrow in each chromatogram is showing the point of mutation. Single peak shows homozygous mutation while double peaks show heterozygous carrier.
(a) Showing peaks of wild and mutant allele in individual IV:5 (b) Showing peaks of wild and mutant allele in individual IV:6 (c) Showing A>C in individual IV:3 (d) Showing A>C in individual IV:2 (e) Showing peaks of wild and mutant allele in individual V:1

Discussion

We are reporting a family having multiple affected individuals suffering from PD in Pakistan with novel missense variant in *FCHSD1* gene. In our study, we performed WES and identified a mutation in one of causative genes, *FCHSD1*, of PD. *FCHSD1* gene mutations seem to be very rare in Asia and no case has been reported from Pakistan. The family had history of PD as shown in pedigree (Fig.1) indicating a possible role of this mutation. All patients experienced from slow progression of disorder. Patients experienced mild gait imbalance, dysphagia, dysarthria, positive Hoffmann sign, rest tremor, diadochokinesia, drooling and dementia with progression of disease as shown in Table 1. None of the patients experienced mental retardation. Age of onset ranges from 20 to 40 in both homozygous carriers. Both of them were not subjected to any treatment in present of near past. No additional features except PD symptoms were present in homozygous patients. None of heterozygous carriers experienced any signs or symptoms of PD.

FCHSD1 gene is involved in synthesis of protein through mTOR signaling pathway [5]. *FCHSD1* gene variants are related with progression of PD through mTOR pathway [6]. A plethora of loci of the candidate genes have been identified which are associated with PD, and this association has

been validated among various populations. However, studies on *FCHSD1* is lacking in many countries of world including Pakistan. Research has shown its involvement in pathogenesis of PD [6].

Risk of development of PD is dependent on age i.e. from childhood to adulthood [29]. Our patients were experiencing cardinal symptoms of PD i.e. bradykinesia, postural instability, muscle rigidity and tremor as previously reported [30]. Our patients experienced similar symptoms as unveiled by previous studies. Our patients also face difficulty in communication as reported that patients suffering from PD often face difficulty to communicate properly i.e. dysarthria, as disease progresses and make it a bit difficult to listeners to understand. Dysphagia was experienced by both patients. Around 35 to 80 % of PD patients suffers from dysphagia with disease progression. Studies show that 8 in 10 patients may suffer from mild to severe dysphagia. This may result in hospitalization, increase in mortality rate and poor quality of life for PD patients [31]. Sialorrhea, also known as drooling, was experienced by both patients. It is reported that around 10 to 84% of PD patients may experience drooling irrespective of ethnic groups [32]. Callabol *et al* suggested that 25 to 30% PD may suffer from dementia [33]. So far, only few cases of *FCHSD1* have been reported and this is the first report of *FCHSD1* gene regarding familial PD.

CONCLUSION

We report a novel missense mutation in *FCHSD1* gene in a consanguineous family from Pakistan causing autosomal recessive early onset Parkinson's Disease. This variant has a potential to be included into the variant database for this disease. In addition to this, this study will help to understand genetic architecture and regulatory mechanism of *FCHSD1* in PD.

LIST OF ABBREVIATIONS

PD = Parkinson's Disease AD: Alzheimer's Disease, SNpc: Substantia nigra pars compacta, WES: Whole Exome Sequencing, LBs: Lewy Bodies, mTOR: mechanistic target of Rapamycin

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Human Ethics committee and the Institutional Bio-safety committee of The Islamia University of Bahawalpur, Pakistan.

HUMAN AND ANIMAL RIGHTS

We hereby affirm that all investigations were conducted in strict accordance with the ethical principles outlined in the Helsinki Declaration.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that all data which is supporting the findings of current research are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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