Genetics of Parkinson's disease in a cohort of Sudanese Population

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Table of Contents

List of abbreviations

ANOVA	Analysis Of Variance
DNA	Deoxyribonucleic acid
PD	Parkinson's disease
NGS	Next Generation Sequencing
TGPS	Targeted Gene Panel Sequencing
WES	Whole Exome Sequencing

1 Abstract

Parkinson's disease (PD) is a progressive neurological disorder that affects various regions of the brain, particularly the substantia nigra (SNr). The prevalence of PD is estimated 1 in 1000 in undisclosed populations, and PD impacts around 1% of individuals over 60 years old. The annual occurrence of PD per 100,000 people ranges from 10 to 20. Currently the diagnosis of PD relies mainly on clinical criteria as there are no cheap and easy available biological markers. It can be challenging to diagnose PD accurately as its characteristic symptoms may also be present in other disorders, making it necessary to conduct further investigations to rule out alternative causes of Parkinsonism.

Studies have suggested a potential genetic connection between disease-causing mutations and risk-associated variants in PD. However, there is limited research in Sudan regarding the role of genetics (and epigenetics) in adult PD. In the studies presented herin, DNA methylation, mutation screening and whole exome sequencing was performed to elucidate the implications of known and candidate genes. DNA from 172 Sudanese individuals, comprising 90 cases and 82 controls, who provided saliva for DNA extraction was analyzed. The average age of onset was 40.6 ± 22.4 years, and 64 patients had a family history of PD. Bisulfite sequencing of a-synuclein (SNCA) intron1 was performed and DNA from 66 individuals, representing 30 families, was analyzed using the Twist custom panel, which allows for screening of 34 genes, 27 risk factors, and 8 candidate qualities associated with Parkinsonism.

To capture the relevant genetic information, the custom Design KAPA Library Preparation Kit from Roche was utilized, targeting exons, intron-exon boundaries, 5'- and 3'-UTR sequences, and 10-bp flanking sequences of the selected genes based on the RefSeq database (hg38 assembly). Whole exome sequencing was conducted using the Illumina NovaSeq 6000 sequencer, achieving a median coverage of 100x and generatig paired-end reads of 100 base pairs. The targeted NGS panel successfully identified both known and novel mutations in six out of thirty-six families, resulting in a diagnostic rate of 20%, consistent with previously reported rates.

Moreover, the average number of diagnosed patients per family using the panel was found to be 3.7 ± 2.3 for families with identified mutations and 1.8 ± 1.5 for unresolved families. This suggests that the targeted gene panel could be a valuable tool for investigating families with two or more affected individuals. Additionally novel variants in candidate genes were discovered in one or more families, which could potentially contribute to disease development or susceptibility to Parkinson's disease.

2. Introduction and aims of the study

2.1 Background

Parkinson disease (PD) is a progressive disorder of the nervous system affecting several regions of the brain, especially the substantia nigra which controls balance and movement. It is the most common movement disorder and the second commonest neurodegenerative disorder after Alzheimer disease. It was first described by James Parkinson in 1817 on his article " Essay on the shaking Palsy" where he meticulously described the cardinal symptoms of the conditions, which are still considered as the hallmark of the disease; Bradykinesia, Rigidity and Tremor (Jost & Reichmann, 2017)

2.1.1 Epidemiology of Parkinson's disease:

PD is considered the second most common neurodegenerative disorder after Alzheimer's disease, however, methodological differences makes it difficult to estimate the exact prevalence of the disease. It is broadly accepted that the prevalence of PD is 1 in 1000 in unidentified populations and it is estimated to affect 1% of the population above 60 years. The annual incidence of PD per 100 000 individuals ranges from 10-20 (Von Campenhausen et al., 2005). With the apparent aging of the global population, PD prevalence is expected to double in the next two decades, accordingly the societal and economic burden of the disease with escalate unless more effective diagnostic, therapeutic and prognostic means are introduced.(Kaltenboeck et al., 2012)

2.1.2 Diagnosis of Parkinson's disease:

The diagnosis of PD is mainly based on clinical criteria, as there is no easily accessible biological marker for the diagnosis up to now The cardinal symptoms and all other non-motor features could be part of disorders that share all these symptoms and more symptoms not related to idiopathic PD. This fact renders the diagnosis of PD a bit complicated as the main symptoms could be demonstrated by taking history and clinical examination but more investigations are required to rule out other causes of Parkinsonism.(Fahn, 2003). Non-motor symptoms are frequent in the early stage of the condition and usually mild in most cases but increase in severity of the disease progression (Zis et al., 2015)

2.1.3 Genetics of Parkinson's Disease

The profile of PD had evolved from the old classic (non-heritable) disease to a multifactorial condition with a profound role of the genetic component. This was uncovered by the involvement of next generation sequencing for familiar type PD, as well as genome wide association studies and candidate gene studies in sporadic PD (Trinh & Farrer, 2013). Familiar cases (monogenic form) of PD are caused by mutations in the PARK2, PINK1, LRRK2, SNCA, or PARK7 genes, or by alterations in genes that have not yet been identified. Alterations in GBA and UCHL1 have been shown to modify the risk to develop the condition (Samii, Nutt, & Ransom, 2004). It appears that there is overlap between the genes that house the disease causing mutations with those of risk associating variants (Table 1)

Table 1: Genes, loci and risk loci involved in monogenic forms of Parkinson's disease (PD), as derived from(Hernandez, Reed, & Singleton, 2016)

Locus	Gene	Protein	Mode of inheritance
Park1	SNCA	a-synuclein	Autosomal dominant
Park2	PARK2	Parkin	Autosomal recessive
Park3	Unknown	Unknown	Autosomal dominant
Park4	SNCA	a-synuclein	Autosomal dominant
Park5	UCHL1	Ubiquitin c terminal hydrolase	Autosomal dominant
Park6	PINK1	Pten-induced putative kinase 1	Autosomal recessive
Park7	PARK7	DJ-1	Autosomal recessive
Park8	LRRK2	Leucine-rich repeat kinase 2	Autosomal dominant
Park9	ATP13A2	Lysosomal type 5 ATPase	Autosomal recessive
Park10	Unknown	Unknown	Risk locus
Park11	GIGYF2	GRB interacting GYF protein 2	Autosomal dominant

Park12	Unknown	Unknown	X-linked
Park13	HTRA2	HTRA serine peptidase 2	Autosomal dominant
Park14	PLA2G6	Phospholipase A2	Autosomal recessive
Park15	FBXO7	F-box only protein 7	Autosomal recessive
Park17	VPS35	Vacuolar protein sorting 35	Autosomal dominant
Park19	EIF4G1	Eukaryotic translation initiation factor 4 gamma 1	Autosomal dominant
-	DNAJC16	DNAJ/HSP40 homolog subfamily C member 6	Autosomal recessive
-	SNCA	a-synuclein	Risk locus
-	LRRK2	Leucine-rich repeat kinase 2	Risk locus
	GBA	Glucocerebrocidase	Risk locus

The treatment approach of PD was directed over the past period into the motor symptoms of the disease and were guided into three main attributes; the pharmacotherapy deep brain stimulation and physiotherapy. Unfortunately motor complications occurs at the late stages of the disease. This suggest that more research should be driven into developing new therapeutics which modify the pathogenicity of the disease.(Oertel & Schulz, 2016)

In Africa, knowledge about the epidemiologic characteristics, pathology and genetics underlying Parkinsonismis sparse. Very few studies have been performed and even those were limited in either sample size or searched variants both which preclude against affirmative generalization and the situation is more profound in Sub-Saharan regions(Okubadejo, Bower, Rocca, & Maraganore, 2006). In North African countries,

mutations in LRRK2 gene were found associated with Parkinson disease in both autosomal dominant and sporadic forms. These results were obtained in Tunisia, Algeria and Morocco. Mutations in PINK1 were associated with early onset PD in an Algerian family (Benamer, de Silva, Siddiqui, & Grosset, 2008)

In Sudan studies that addressed the genetic role in adult PD are scant; in a large complex family mutations in PINK1 were found in early onset PD (Cazeneuve C, 2009), However neither population study nor multifamily study have been done and the genetics of PD in Sudanese families is yet to be discovered. The diverse genetic background in the Sudanese population and the high rate of consanguineous marriages might increase the chance to discover novel variants and even new genes. This was proved by Elsayed et al. (2016) who discovered a novel mutation in juvenile onset PD in a peculiar Sudanese family (Elsayed et al. 2016). The aim of this study was to increase knowledge of the genetic basis of Parkinsons's disease in a cohort of Sudanese individuals, to allow the study of the effects of the identified genetic variants on the structure and function of their respective proteins and to screen for these variants in the Sudanese PD cohort

2.2 Materials and Methods :

2.2.1 Criteria of phenotypic assessment:

Clinical Diagnosis of PD was made using the United Kingdom Parkinson's Disease Society Brain Bank criteria. Upon confirming the clinical diagnosis, cases of PD were examined using the Motor Disorders Society's criteria for Parkinson's disease (MDS-UPDRS). The study was approved by the Research Ethics committee for Medical and Health studies at the faculty of medicine, University of Khartoum. All participants signed informed consent. To determine familial cases we considered positive family history and/ or parental consanguinity A total of 172 individuals (66 patients from 30 families plus 23 idiopathic cases with matching controls) donated 1-2 ml saliva using Oragene Discover as a primordial tissue for DNA, which was extracted utilizing prepIT ® L2P manual protocol. DNA quality and quantity were performed using agarose gel electrophoresis and spectrophotometry (Nanodrop®, Thermoscientific).

2.3 Genetic and epigenetic testing:

2.3.1 Methylation of alpha- synuclein (SNCA):

We screened all index individuals for methylation state of SNCAintron1, details of the analysis are available in the result section of the first publication.

2.3.2 Target Panel Next Generation Sequencing: (TPNGS)

DNA from 66 individuals representing 30 families were sent to Targeted Panel NGS.We planned Twist custom panel, which permits screening of 34 qualities, 27 chance components and 8 candidate qualities, which are for the most part related to Parkinsonism. The custom Design KAPPA Library Preparation Kit (Roche) was utilized to capture all exons, intron– exon boundaries, 5′- and 3′-UTR sequences and 10bp flanking sequences of target genes (RefSeq database , hg38 assembly). Specific probes for NGS target enrichment were designed using NimbleDesign1 software and amplicon length var¬ied between 250 and 500 bp. Runs were performed on Illumina MiSeq sequencer. The test was performed according to manufacturer's recommended protocol. Variants were prioritized based on the following criteria: frequencies <0.01% in open databases (ExAC/ GnomAD) and our in-house database of 500 exomes, nucleotid and amino-acid conservation (based on alignments), relation of the quality to disease (per family),and inheritance pattern.

Bioinformatics analysis of gene Panel Data:

Human reference genome UCSC hg1 was utilized for sequence alignment and variant calling with the Burrows-Wheeler Aligner (BWA) (Li H & Durbin, 2010) and the Genome Analysis Toolkit (GATK).

https://design.nimblegen.com/nimbledesign/app/.2http://bio-bwa.sourceforge.net/.

3http://www.acronymfinder.com/Genome-Analysis-Toolkit-(software)-(GATK).html.

PCR duplicates were removed prior to variant calling using Picard4 program. Variants were annotated with ANNOVAR software (Li H & Durbin, 2010). The mean coverage was 993× (range 594–1241×), and the mean percent coverage at 30× was 98.7% (range 96.5–99.6%) for all individuals tested.

2.3.3 Whole Exome Sequencing:

DNA from 43 from 30 families were sent for whole Exome Sequencing (WES), who represented the unsolved cases from the targeted panel NGS.

Library preparation: We utilized Human Core Exome kit in combination with the RefSeq Panel kit (Twist Bioscience, San Francisco, USA), which was utilized for the enrichment of the exome. Description of capturing all exonic regions in the following link:https://wwwtwistbioscience.com/resources/data-files/human-core exomerefseq-panel-bed-files).

The whole exome sequencing was carried out at the University Hospital Bonn NGS core facility using Illumina NovaSeq 6000 sequencer (San Diego, California, United States), which produces a median coverage of 100x and pair ended read of 100 base-paired.

2.3.4 Bioinformatics analysis:

The resulting high quality paired reads were aligned using Burrows–Wheeler (BWA-MEM) algorithm v0.7.12 with default parameters to human reference genome hg38. (H. Li & Durbin, 2010). The resulting BAM files were processed for removing any PCR dublicates, sorting and indexing files using samtools v1.7. Variant calling was performed using Freebayes v1.0.0 (Garrison & Marth, 2012) with default parameters, and the resulting VCF files according to depth (>20) and genotype quality (>20). As such, the processed VCF files were annotated and priortised using VarAFT annotation and filtered tools. Variants were filtred according to minor allele frequency cut-off of 0.01 in the GnomAD genome database, the variant's effect and in-silico prediction pathogenicity tools. For the first part, the initial focus was on the variants with major structural effects. Then attention was toward missense variants, which were annotated as pathogenic by SIFT and PolyPhen softwares, in addition to the in frame variants. Both Online Inheritance in Man (OMIM) disease-related genes (https://wwwomim.org/) and Orphanet database (https://wwworpha.net/consor/cgi-bin/index.php) were checked for the associating genes The resulting variants status were examined as per the American College of Medical Genetics and Genomics guidelines ACMG (Bathesda, United states). Only variants of unknown significant, likely pathogenic or pathogenic confirmed using Variant Effect Predictor VEP, available in the link (https://wwwensembl.org/info/docs/tools/vep/index.html) and pathogenic variants were amplified and sequenced using Sanger sequencing to comfirm segregation

Segregating variants were checked in the Sudanese exome, which contains 131 exo-mes were we estimated allele frequency and possible Identical by Decent sharing (IBD) to role out population polymorphism. .

2.4 References:

Benamer, H. T. S., de Silva, R., Siddiqui, K. A., & Grosset, D. G. (2008). Parkinson's disease in Arabs: A systematic review. Movement Disorders, 23(9), 1205–1210. https://doi.org/10.1002/mds.22041

Cazeneuve, C., Sean, C., Ibrahim, S. A., Mukhtar, M. M., Kheir, M. M., LeGuern, E., … Salih, M. A. (2009). A new complex homozygous large rearrangement of the PINK1 gene in a Sudanese family with early onset Parkinson's disease. Neurogenetics, 10(3), 265–270. https://doi.org/10.1007/s10048-009-0174-4

Elsayed, L. E. O., Mohammed, I. N., Hamed, A. A. A., Elseed, M. A., Salih, M. A. M., Yahia, A., … Stevanin, G. (2018). Case report of a novel homozygous splice site mutation in PLA2G6 gene causing infantile neuroaxonal dystrophy in a Sudanese family. BMC Medical Genetics, 19(1), 1–5. https://doi.org/10.1186/s12881-018-0592- y

Fahn, S. (2003). Description of Parkinson' s Disease as a Clinical Syndrome. Annals New York Academy of Sciences, 991, 1–14. https://doi.org/10.1111/j.1749-6632.2003.tb07458.x

Garrison, E., & Marth, G. (2012). Haplotype-based variant detection from short-read sequencing. 1–9. Retrieved from http://arxiv.org/abs/1207.3907

Gelb, D. J., Oliver, E., & Gilman, S. (1999). Diagnostic criteria for Parkinson disease. Archives of Neurology, 56(1), 33–39. https://doi.org/10.1001/archneur.56.1.33

Goetz, C. G., Tilley, B. C., Shaftman, S. R., Stebbins, G. T., Fahn, S., Martinez-Martin, P., … Zweig, R. M. (2008). Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Scale presentation and clinimetric testing results. Movement Disorders, 23(15), 2129–2170

Hernandez, D. G., Reed, X., & Singleton, A. B. (2016). Genetics in Parkinson disease: Mendelian versus non-Mendelian inheritance. Journal of Neurochemistry, 139, 59–74. https://doi.org/10.1111/jnc.13593

Jost, W. H., & Reichmann, H. (2017). "An essay on the shaking palsy" 200 years old. Journal of Neural Transmission, 124(8), 899–900. https://doi.org/10.1007/s00702-

017-1684-0

Kaltenboeck, A., Johnson, S. J., Davis, M. R., Birnbaum, H. G., Carroll, C. A., Tarrants, M. L., & Siderowf, A. D. (2012). Direct costs and survival of medicare beneficiaries with early and advanced parkinson's disease. Parkinsonism and Related Disorders, 18(4), 321–326. https://doi.org/10.1016/j.parkreldis.2011.11.015

Li, H., & Durbin, R. (2010). Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics, 26(5), 589–595. https://doi.org/10.1093/bioinformatics/btp698

Oertel, W., & Schulz, J. B. (2016). Current and experimental treatments of Parkinson disease: A guide for neuroscientists. Journal of Neurochemistry, 139, 1–13. https://doi.org/10.1111/jnc.13750

Okubadejo, N. U., Bower, J. H., Rocca, W. A., & Maraganore, D. M. (2006). Parkinson's disease in Africa: A systematic review of epidemiologic and genetic studies. Movement Disorders, 21(12), 2150–2156. https://doi.org/10.1002/mds.21153

Samii, A., Nutt, J. G., & Ransom, B. R. (2004). Parkinson's disease. Lancet (London, England), 363(9423), 1783–1793. https://doi.org/10.1016/S0140-6736(04)16305-8

Trinh, J., & Farrer, M. (2013). Advances in the genetics of Parkinson disease. Nature Reviews. Neurology, 9(8), 445–454. https://doi.org/10.1038/nrneurol.2013.132

Von Campenhausen, S., Bornschein, B., Wick, R., Bötzel, K., Sampaio, C., Poewe, W., … Dodel, R. (2005). Prevalence and incidence of Parkinson's disease in Europe. European Neuropsychopharmacology, 15(4), 473–490. https://doi.org/10.1016/j.euroneuro.2005.04.007

Zis, P., Martinez-Martin, P., Sauerbier, A., Rizos, A., Sharma, J. C., Worth, PF., … Chaudhuri, K. R. (2015). Non-motor symptoms burden in treated and untreated early Parkinson's disease patients: Argument for non-motor subtypes. European Journal of Neurology, 22(8), 1145–1150. https://doi.org/10.1111/ene.12733

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Short communication

Methylation of alpha-synuclein in a Sudanese cohort

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ABSTRACT

Background: Several studies suggested a significant role of epigenetic changes, including alterations in miRNA, histone modifications, and DNA methylation of α-synuclein (*SNCA*) in Parkinson's disease (PD) pathogenicity. As of yet, only very few studies have been carried out in this field in Africa and none in Sudan.
Materials and methods: We collected DNA from 172 Sudanese individuals (90 cases, 82 controls) who donated saliva for DNA extraction (mean age of onset: 40.6 22.4 years). A family history of PD was evident in 64 patients. DNA preparation and bisulfite sequencing of $SNCA_{intron1}$ was performed as described earlier.
Results: Of the fourteen analyzed CpGs of $SNCA_{intron1}$, CpGs 16-23 were hypomethylated in PD (P-value ranged from 0.023 to 0.003). P–values improved, when sporadic cases were excluded from the analysis.
Conclusion: We identified the presence of a specific pattern of DNA methylation in a young Sudanese cohort of familial PD, which confirms the importance of the methylation of $SNCA_{intron1}$ for PD. This phenomenon appears to be independent of ethnicity, the impact of environmental factors, drug history, or familial clustering.

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1. Introduction

Most Parkinson's disease (PD) cases appear to be idiopathic (85-90%) and clear-cut identified mendelian genetic factors are involved in only 10-15% of cases [1]. Among the genes associated with PD, *SNCA* which encodes the α-synuclein protein (α-syn), is considered a major contributor to PD pathogenicity. α-syn is a component of Lewy bodies, the pathological hallmark of PD [2]. It has been hypothesized, that aggregates of misfolded α-syn may spread in the brain and the amount of α-syn is considered a major determinant of disease severity, thus its expression and regulation are important risk factors for PD [3,4]. *SNCA* expression is linked to $SNCA_{intron1}$ methylation [5,6] and subsequent sequestration of DNA methyltransferase 1 (DNMT1) from the nucleus to the cytosol [7].

In Africa, little is known about the putative environmental influence on PD incidence, pathogenesis, and genetic predisposition. Genomic diversity, together with the various cultural and environmental features of the African population poses considerable challenges [8]. The current difficult political and social situation in sub-Saharan Africa (SSA) in additional hampers essential research activities. Here we present the first epigenetic study of *SNCA* in a Sudanese cohort, focusing on methylation of $SNCA_{intron1}$.

2. Material and methods

A cohort of Sudanese PD patients with a positive family history of PD and additional sporadic cases were investigated. The clinical diagnosis of PD was clinched as per the criteria of the UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria [9]. Confirmed clinically diagnosed cases of PD were examined using the Motor Disorders Society's criteria for Parkinson's disease (MDS- UPDRS). The study was approved by the Research Ethics committee for Medical and Health studies at the faculty of medicine, University of Khartoum. All participants signed informed consent.

172 individuals (90 PD, 82 controls) donated Saliva for DNA extraction, with an average mean age of onset 40.6 22.4 years, an average age of 52.7 19.1 years and the male to female ratio is 1.65.

One to 2 mL of saliva were collected using Oragene.Discover® DNA collection kits (Genotek Inc.). DNA purification was done according to the prepIT®.L2P manual protocol provided by the manufacturer (http://www.dnagenotek.com/US/products/OGR500.html). DNA quality and quantity control were performed using standard Agarose gel electrophoresis as well as spectrophotometry (NanoDrop®, Thermo Scientific).

2.1. Bisulfite-specific polymerase chain reaction

Bisulfite treatment was performed with 500 ng DNA and EZ-96 DNA Methylation-Gold Kit (Zymo Research®, Freiburg im Breisgau); CpG positions were numbered as previously described [6]. For polymerase chain reaction (PCR) 1 (CpGs 2-7), 2 μL bisulfite-treated DNA was amplified by using 5.5 pmol primers each (SYN-F1a_GGAGTTTAAKGAAAGAGATTTGATT and SNCA-PR2a/Bio_Bio-CTCCYTACACTTCCATTTCATTATTTAC) and 15 μl HotStarTaq Plus Mastermix (Qiagen, Hilden) in a 30 μl reaction: 95 C for 5 min, 50x (95 C for 35 s, 48 C for 35 s, 72 C for 35 s), 72 C for 5 min. For PCR 2 (CpGs 16-23), 1 μL bisulfite-treated DNA was amplified by using 5 pmol primer each (SNCA PF1_ GTTTGGTAAATAATGAAATGGAAGTGTA and SNCA-PR8/Bio_Bio-CCAAATATAATAATTCTAATCCATC) and 12.5 μl HotStarTaq Plus Mastermix (Qiagen) in a 25 μl reaction: 95 C for 5 min, 47x (95 C for 40 s, 56 C for 40 s, 72 C for 40 s), 72 C for 5 min.

2.2. Bisulfite pyrosequencing

We used 20–25 μl bisulfite-specific PCR reaction, 5 pmol primer SNCA-PS11 (TTGAGAGATTAGGTT GTT), and SNCA-PS2 (GTTTTTTYG GGTGGTT), respectively, and the Pyromark Gold Q24 Reagents (Qiagen) to perform sequencing on a Pyromark Q24 (Qiagen), as reported previously.

2.3. Statistical analysis

To compare the degree of methylation for each CpG between healthy and PD patients, we used one-way ANOVA.

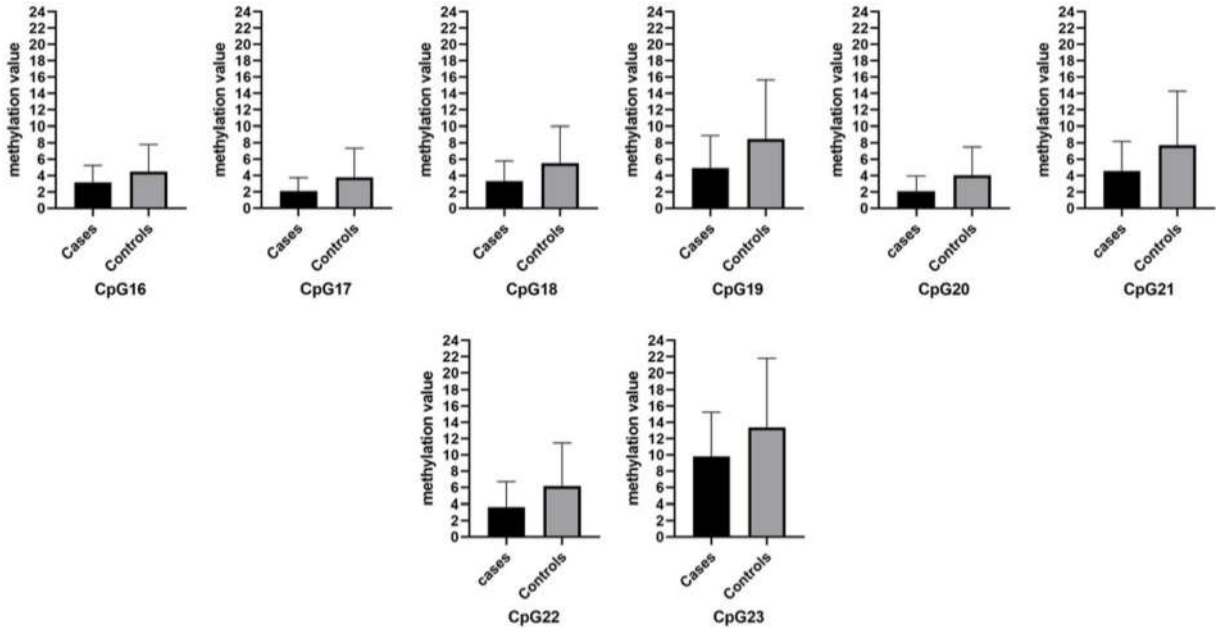

Fig. 1. Methylation of $SNCA_{intron1}$ in Parkinson's patients and controls. CpGs 16-23 are significantly hypomethylated in PD patients when compared to healthy individuals (p- value at least 0.05).

Table 1
P-value for CpGs 16-23 methylation in both total and familial cohorts.

P-value	CpG 16	CpG 17	CpG 18	CpG 19	CpG 20	CpG 21	CpG 22	CpG 23
Total cohort	0.023363	0.003395	0.003338	0.002977	0.000812	0.003996	0.004767	0.013863
Familial cohort	0.003345	0.000146	0.000123	0.000242	2.16E-05	3.64E-05	4.78E-05	0.000661

The total of 172 individuals with 68 familial PD with their 62 controls form their families, 23 sporadic cases with their 19 matching controls. PD: Parkinson's disease.

2.4. Availability of data

All data used or generated from this study are available from the corresponding authors upon justifiable request.

3. Result

Methylation of *SNCA*$_{intron1}$ was analyzed using two pyrosequencing reactions covering two regions (CpGs 2-7 and CpGs 16-23), according to Schmitt et al., 2015(10). CpGs 2-7 were hypomethylated in the Sudanese proband, however, this association was not statistically significant (p-values: 0.81, 0.71, 0.50, 0.48, 0.90, 0.24 respectively). CpGs 16-23 were hypomethylated significantly (Fig. 1 and Table 1); a separate analysis of the familial PD cohort (cases controls, n 130) confirmed these results (Table 1).

We used the Bonferroni test to adjust for multiple comparisons, we found α to be 0.00625. CpG 16 and 23 fall behind the new α, whereas all p-values remain statistically significant for the familial cohort.

4. Discussion and conclusion

We found the specific pattern of DNA methylation in *SNCA*$_{intron1}$, which has been described earlier in a cohort of European ethnicity also in our young Sudanese population cohort, which points to the importance of this phenomenon for PD. Reduced methylation of *SNCA*$_{intron1}$ appears to be independent of ethnicity, familial clustering, and impact of environmental factors, including medication. Hypomethylation of CpGs 2-7 was not statistically significant in our Sudanese cohort, which is nondescript when compared with previous results utilizing the similar technique, including own previous work [10].

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Authors contribution

Conception and design: UW, IS, YB, OS, LEOE; Primary clinical evaluation: OS, EB, HB, FY, AH, EAAI, INM, SME, MAE. Secondary clinical assessment (UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria AND MDS-UPDRS) participant recruitment and sampling: YB, MAA, MTS, MOI, AAE, KE, HAA, YAI, MEA, RA, MAA, AAA; SME, MAO, AAM, MYT, SMM, SHE, RJK, MTM, AA. DNA preparation and PCR: YB: Pyrosequencing: YB IS. Data analysis and interpretation: YB, IS, and UW. Writing the first draft: YB. Witing with critical revision: YB, UW, IS, MAE, LEOE: All authors revised the secondary version and approved the final version of the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

References

[1] A. Verstraeten, J. Theuns, C Van Broeckhoven, Progress in unraveling the genetic etiology of Parkinson's disease in a genomic era, Trends Genet. [Internet] 31 (3) (2015) 140–149, https://doi.org/10.1016/j.tig.2015.01.004. Available from.
[2] A. Osterhaus, J. Groen, M. Bildt, De Van, B. Martina, J. Vos, H Van Egmond, Î -Synuclein in Lewy bodies, Nature 388 (1997) 839–840.
[3] J. Li, E. Englund, J.L. Holton, D. Soulet, P. Hagell, A.J. Lees, et al., Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation, Nat. Med. 14 (5) (2008) 501–503.
[4] L.A. Volpicelli-daley, K.C. Luk, V.M. Lee, Addition of exogenous a-synuclein preformed fibrils to primary neuronal cultures to seed recruitment of endogenous a-synuclein to Lewy body and Lewy neurite–like aggregates, Nat. Protoc. 9 (9) (2016), 9.
[5] L. Matsumoto, H. Takuma, A. Tamaoka, H. Kurosaki, H. Date, S. Tsuji, CpG demethylation enhances alpha-synuclein expression and affects the pathogenesis of Parkinson's disease, PLoS One 5 (11) (2010) 1–9.
[6] A. Jowaed, I. Schmitt, O. Kaut, U. Wu, Methylation regulates alpha-synuclein expression and is decreased in Parkinson's disease patients'brains, J. Neurosci. 30 (18) (2010) 6355–6359.
[7] P. Desplats, B. Spencer, L. Crews, P. Pathel, D. Morvinski-friedmann, K. Kosberg, et al., α-Synuclein induces alterations in adult neurogenesis in Parkinson disease models via p53-mediated repression of, J. Biol. Chem. [Internet] 287 (38) (2012) 31691–31702, https://doi.org/10.1074/jbc.M112.354522. Available from:.
[8] L. Pereira, L. Mutesa, P. Tindana, M. Ramsay, African Genetic Diversity and Adaptation Inform a Precision Medicine Agenda, Nat Rev Genet [Internet], 2021; 22(May, https://doi.org/10.1038/s41576-020-00306-8. Available from:.
[9] S. Article, Diagnostic criteria for Parkinson's disease, Arch. Neurol. 56 (2021) 33–39.
[10] I. Schmitt, O. Kaut, H. Khazneh, A. Ahmad, D. Berg, L-dopa increases α -synuclein DNA methylation in Parkinson's disease patients in vivo and in vitro, Mov. Disord. 30 (13) (2015) 1794–1801.

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Parkinsonism and Related Disorders

Short communication

Intrafamilial and interfamilial heterogeneity of *PINK1*-associated Parkinson's disease in Sudan

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ABSTRACT

PINK1 is the second most predominant gene associated with autosomal recessive Parkinson's disease. Homozygous mutations in this gene are associated with an early onset of symptoms. Bradykinesia, tremors, and rigidity are common features, while dystonia, motor fluctuation, and non-motor symptoms occur in a lower percentage of cases and usually respond well to levodopa. We investigated 14 individuals with parkinsonism and eleven symptom-free siblings from three consanguineous Sudanese families, two of them multigenerational, using a custom gene panel screening 34 genes, 27 risk variants, and 8 candidate genes associated with parkinsonism. We found a known pathogenic nonsense *PINK1* variant (NM_032409.3:c.1366C

Abbreviations: PD, Parkinson's disease; AREOPD, Autosomal recessive early onset Parkinson's disease; AOO, Age of onset; ACMG, American College of Medical Genetics and Genomics; HGVS, Human Genome Variation Society.

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1. Introduction

Approximately 3–5% of all Parkinson's disease (PD) patients suffer from a monogenic disease, caused by dominant mutations in the alpha-synuclein (*SNCA*), (*LRRK2*), (*VPS35*), or (*EIF4G1*) genes, or by recessive mutations in the parkin (*PRKN*, *PARK2*), *PINK1* (PARK6), *DJ-1* (PARK7), *ATP13A2* (PARK9), *PLA2G6* (PARK14) and *FBXO7* (PARK15) genes, as well as mutations in the *SYNJ1*, *DNAJC6* and *VPS13C* genes. The recessive diseases in general present with younger onset, higher incidence of non-motor symptoms, and alternative atypical pathology [1].

PRKN and *PINK1* are the most prominent genes associated with autosomal recessive PD [1]. Mutations in these two genes are associated with autosomal recessive early onset PD (AREOPD) and a significant proportion of patients presents with juvenile onset (

of the lower limbs) is not common in *PINK1*-associated parkinsonism. Most patients respond well to levodopa treatment [2].

Mutations in *PINK1* have been reported in many regions across the world, mostly from Europe, North America, North Africa [3], the Middle East [4], and South Africa [5]. *PINK1* mutations and complex rearrangement were also found in two consanguineous Sudanese families [6,7]. We here present a multiple-family cohort with novel and known pathogenic variants associated with *PINK1*-related AREOPD as well as juvenile-onset parkinsonism with intra-familial and interfamilial variability in large multigenerational Sudanese families.

2. Patients and methods

The Research Ethics committee for Medical and Health studies at the Faculty of Medicine, University of Khartoum, approved this study. All participants gave informed consent. Clinical diagnosis of PD was made using the United Kingdom Parkinson 's Disease Society Brain Bank criteria and secondary parkinsonism was excluded; the family histories were negative for essential tremor and Alzheimer's disease. PD patients were characterized using the Movement Disorders Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) and video recordings of the clinical assessment were saved for future reference. All patients were assessed in the morning before taking antiparkinson medications (i.e. 12-14 h without PD medication). Twenty-four individuals from three consanguineous, multi-generational Sudanese families donated saliva samples for DNA extraction (fourteen patients and nine symptom-free siblings, plus a single patient from consanguineous parents) which were collected using Oragene Discover® collection kits. DNA purification was carried out following the prepIT ® L2P manual protocol. DNA quality and quantity were checked using agarose gel electrophoresis and spectrophotometry (Nanodrop®, ThermoFisher Scientific).

We utilized a custom gene panel (Twist Bioscience) which allowed screening of 34 genes, 27 risk variants, and 8 candidate genes associated with parkinsonism (panel details are available in Supplementary Table S1). The custom Twist EF Library Prep was used to capture all exons, intron–exon boundaries, 5 - and 3 -UTR sequences, and 10-bp flanking sequences of target genes (RefSeq database, hg38 assembly). Specific probes for NGS target enrichment were designed using Twist software and amplicon length varied between 250 and 500 bp. Sequencing runs were performed on Illumina MiSeq sequencer. The assay was performed according to the manufacturer's recommended protocol. Human reference genome UCSC hg38 was used for sequence alignment and variant calling with the Burrows-Wheeler Aligner (BWA; http://bio-bwa.sourceforge.net/) and the Genome Analysis Toolkit (GATK; https://gatk.broadinstitute.org/). PCR duplicates were removed prior to variant calling using Picard software (https://broadinstitute.github.io/picard/). The mean coverage was 993 (range 594–1241), and the mean percent coverage at 30 was 98.7% (range 96.5–99.6%) for all individuals tested. Variants were annotated with ANNOVAR software (https://annovar.openbioinformatics.org/).

Variants were prioritized based on the following criteria: frequencies

500 exomes, nucleotide and amino-acid conservation (based on alignments), the relation of the gene to disease (per family) and inheritance pattern. Filtered variants were amplified with conventional PCR; primers were designed using Primer3 (https://primer3.ut.ee/) as follows: PD005: Forward: GGCTTTGGGTTGAGACTGTG, Reverse: CTTCTCTGTGAGCCTGTTGG; PD013: Forward: TCAATCCCTTC-TACGGCCAG, Reverse: GGATCTGTCACTGTGGCTCT; PD016 Forward: GCTTCTTATTAGGGCTGGCA, Reverse: TGCAGTCCCTTTCCCAAATG. The variants were then sequenced using Sanger sequencing to confirm the mutations and analyze segregation. Sanger sequences alignment was performed using Bioedit software v7.2.5 using default parameters. The variants are presented in the text of this paper using HGVS format. HGVS variant descriptions were normalized using Mutalyzer (https://mutalyzer.nl).

3. Results

Fourteen patients in three consanguineous families (PD005, PD013, and PD016) were diagnosed with AREOPD, as shown in the pedigrees (Fig. 1). The mean age of onset (8.4 years and the female-to-male ratio was 0.422. Two of the three families had multiple affected family members. In PD005, 13 family members in the last four generations had a history of parkinsonism (six males and four females, two deceased females and one deceased male); eight of the 10 living patients took part in the study with an average age of onset (AOO) of 25.1 8.7 years (Fig. 1A). Family PD013 had five patients (three males and two females) in the index generation, all of whom took part in the study, with an average AOO of 30.2 years (Fig. 1B). The third family, PD016, had one patient in the last generation (AOO: 30 years), and the parents are consanguineous (Fig. 1C).

Bradykinesia and limb rigidity were prevalent in all patients, who displayed a wide range of disease severity of symptoms (Table 1). Forty seven percent had resting tremor and 70.6% showed variable degrees of postural instability. All patients were levodopa responsive except individual PD005F (Table 1). Patient PD013G was uncooperative when asked to perform parts of the examination that required articular actions. No signs of other movement disorders were observed in any of the patients.

Two novel frameshift variants were identified, in addition to known pathogenic nonsense and frameshift variants in *PINK1*. We identified a novel homozygous frameshift insertion NM_032409.3: c.1448_1449insTCAGTGCCTCCAGACTTGAG; p. (Arg483Serfs*7) (Normalized HGVS description: NM_032409.3:c.1448_1449ins [1429_1443; TTGAG]; ACMG classification: pathogenic) in two members of family PD013 (PD013A and PD013B) who represent two of the three branches in the family (Fig. 1B). The patient PD013G, who represents the third branch, had a substantially higher AOO (38 years) and did not carry the variant. Another novel homozygous frameshift variant NM_032409:c.1597dup; p.(Gln533Profs*29) (ACMG classification: pathogenic) was found in three probands – PD005A, PD005K and PD005E – who represent three of the four branches in family PD005 esequencing confirmed the presence of the variant in the homozygous state also in this individual. In the family PD016 we found the known homozygous stop-gain variant NM_032409.3:c.1366C

Sanger sequencing confirmed the segregation of these variants in all probands (including PD005F that did not show the variant in the panel screening) except PD013G who is likely a phenocopy (Supplementary Fig. S1F). These protein truncating variants are predicted to lead to nonsense mediated decay. The variants were classified as pathogenic based on ACMG criteria PVS1 (null variants), PM2 (absent from controls), and PP1 (segregation).

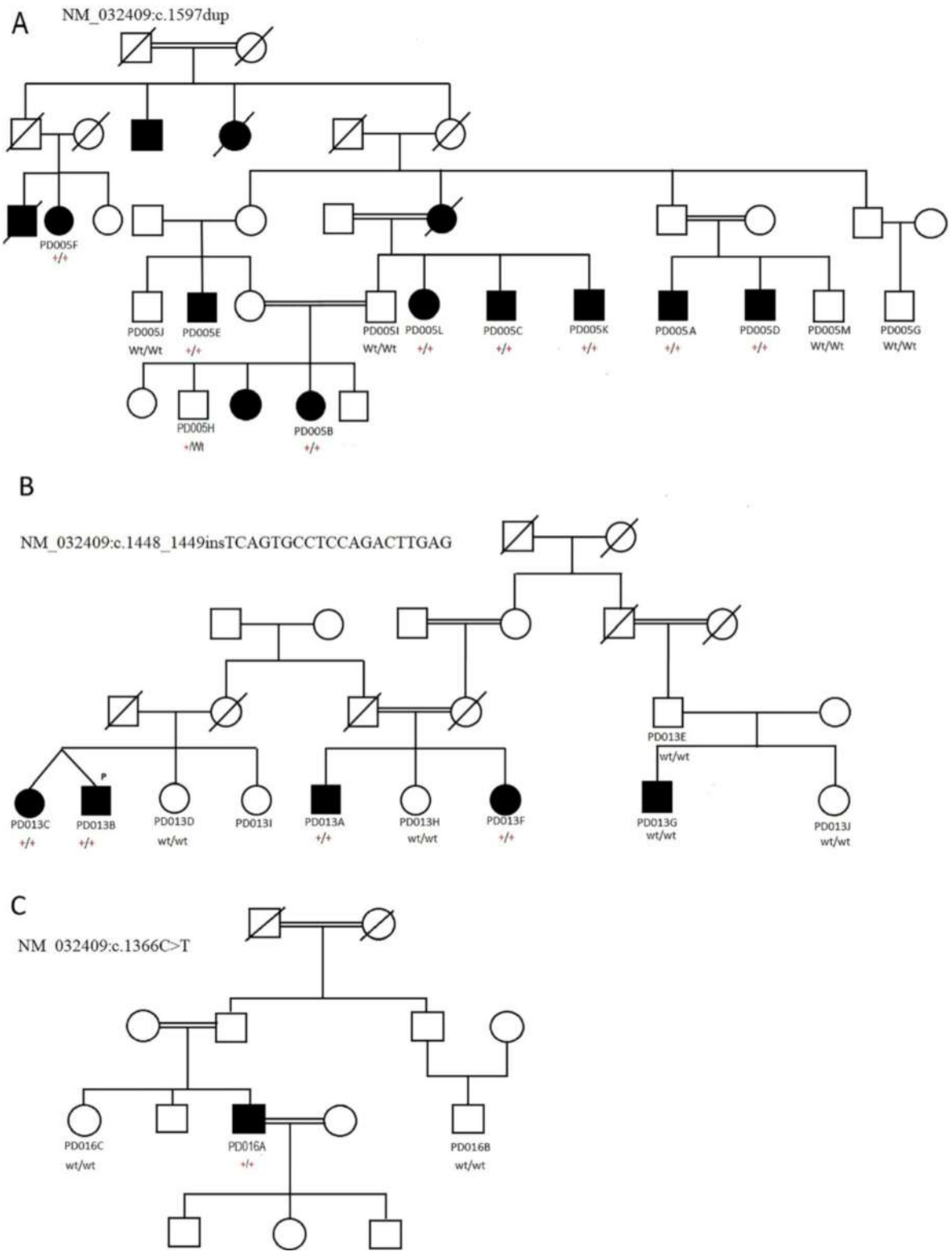

Fig. 1. Heredogram showing three families with PINK1-related autosomal recessive Parkinsonism, each with a different mutation and a showcase of cosegregation. **A**: the segregation analysis of the variant NM_032409:c.1597dup, Which is found to be homozygous in all family probands, heterozygous in the control individual PD005H, and absent from all symptoms-free controls. **B**: Segregation analysis of the variant NM_032409:c.1448_1449insTCAGTGCCTCCAGACTTGAG, which is absent from all symptoms-free controls plus the patient PD013G, and found as homozygous in the rest of the family proband. **C**: Segregation analysis of the family PD016 showing the homozygous variant NM_032409:c.1366C

Table 1
Summary score of motor and non-motor features of the Sudanese PD patients.

Patient's code	Sex	Age of onset (years)	Age at examination (years)	Bradykinesia (in the most affected limb) (0–4)	Postural instability (0–4)	Rigidity (in the most affected limb) (0–4)	Resting Tremor (in the most affected limb) (0–4)	Hoehn and Yahr stage (0–5)	Depression / Anxiety	Cognitive impairment
PD005-A	M	19	40	1	0	1	3	2	0	0
PD005-B	F	25	57	1	0	2	0	2	0	0
PD005-C	M	27	56	3	3	3	4	4	0	1
PD005-D	M	21	56	1	1	2	3	3	0/1	0
PD005-E	M	19	34	2	0	3	2	2	0	0
PD005-F	F	39	60	4	4	3	0	5	1/1	0
PD005-K	M	13	43	2	3	3	0	3	1/1	0
PD005-L	F	38	63	3	0	2	1	2	2/1	1
PD013-A	M	30	60	3	4	3	3	5	1/1	1
PD013-B	M	22	52	1	1	2	1	3	1/1	1
PD013-C	F	40	52	2	1	2	0	3	0	0
PD013-F	F	23	54	2	1	1	0	3	1/1	0
PD013-G	G	36	41	4	4	2	0	5	3/3	0
PD016-A	M	30	58	4	4	4	0	4	4/3	0

Scores are compiled from MDS-UPDRS data of the patients.
Note: The first part of the patient code (PD plus the number) denotes the particular family of interest, while the following letter denotes the particular individual in the family; e,g; PD005A Individual A in the family PD005.

Discussion

This study identified pathogenic *PINK1* variants in three consanguineous Sudanese families. Two of the variants are novel and all are protein truncating, thus predicted to cause loss of function – the known mechanism of disease pathogenesis.

The novel *PINK1* variant (p.Gln533Profs*29) resulted in frameshift insertion segregating in family PD005. Family PD005 to our knowledge is the largest family in terms of the number of probands with *PINK1*-associated AREOPD; 13 individuals in four generations are affected. The homozygous variant is shared among all eight sampled probands including one proband (PD005F) that showed a reference genotype in the gene panel screening (Fig. 1A). Thus, gold-standard Sanger sequencing superseded targeted next generation sequencing in confirmation and segregation analysis. Clinically, the parkinsonian features presented were similar to those reported in previous studies, with prevailing bradykinesia and rigidity among all patients [6,7]. Interestingly, notable phenotypic variability is present within this family: PD005K had a juvenile-onset disease, while his two siblings PD005C and PD005L developed the first symptoms in adulthood (Fig. 1A and Table 1). Considerable variability in the severity of symptoms was observed among them. Only PD005F showed a poor response to levodopa (and symptoms of developmental hearing impairment). All probands had been living in the same households in their villages throughout their lives, which renders additional external environmental effects unlikely an explanation for the intrafamilial heterogeneity. We identified a second novel variant (p.Arg483Serfs*7) in family PD013. The variant co-segregated in a homozygous state in two branches (PD013A and PD013B), but not in a third one (PD013G). The later age at onset (38 years) compared to the family average (30 years) and the unique motor features suggested that PD013G does not have the same disease as the other family members (phenocopy), and indeed did not carry this insertion (Fig. 1B). Last, we found a known pathogenic variant (p.Gln456*) in family PD016, which was present in a homozygous state in the affected individual while absent from the symptoms-free relatives (Fig. 1C). This nonsense variant was reported for the first time in an Italian family with a similar course of disease progression except that the current patient has a history of dystonia and hallucinations, which were not reported in the Italian family [8]. Several studies have described cases of Arab-Berber in North Africa as well as Caucasian European and Caucasian Canadian who carry the same variant, but this is the first time to report the variant in a family with sub-Saharan African descent. The variant was homozygous in most previously reported probands, except in two cases (p.Gln456* *trans* p.Cys125Gly and p.Gln456* *trans* p.Ala383Thr) [9]. A comparison with the phenotypic data of the Sudanese probands from previous reports is detailed in Supplementary Table S2.

The phenotypic heterogeneity of *PINK1*-associated PD in this cohort likely reflects the peculiarities of the Sudanese population that has one of the largest genetic pools in the world [10]. The high genetic diversity and effective population size increase the chance of novel phenotypic and genotypic findings. *PINK1*-related autosomal recessive PD has been identified in Sudanese kindreds in previous studies. Interestingly, the average age of onset in all Sudanese kindreds (range 11–40 years), is below what has been described in general [9], as more juvenile cases were found in these Sudanese families (12.6 1.9 in a study by Anne-Louise Leutenegger et al. [6], 15.7 4.1 in another study by C 8.4 in our study). The uniqueness of the Sudanese population was also illustrated in a previous study, where we described specific patterns of DNA methylation of *SNCA* intron1 in familial cases of PD compared to symptom-free controls from Sudan [11]. Despite the limited size of the current cohort and the previous *PINK1*- associated PD families from Sudan, we could assume that *PINK1* is more common than *PRKN* unlike the incidence of AREOPD in Europe, this could be a coincidence or due to the difference in the populations. Further investigation of Sudanese PD patients with detailed clinical assessment, social and demographic assessment, and high throughput genetic testing will help to understand the phenotype-genotype correlation of PD in Sudan.

Additional data

Additional data that support the findings of this study are available from the corresponding author upon reasonable request.

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Declaration of interest

- The authors have no relevant financial or non-financial interests to disclose.

Authors contribution

Conception and design: UW, JCC, YB, OS, SL, LEOE; Primary clinical evaluation: OS, Secondary clinical assessment (UK *Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria AND MDS-UPDRS) participant recruitment and sampling: YB, MAA, MTS, MOI, AAE, KE, HAA, YAI, MEA, RA, MAA, AAA; SME, MAO, AAM, MYT, SMM, SHE, RJK, MTM, AA. DNA preparation and PCR: YB: Panel sequencing: YB, SL, CT. Data analysis and interpretation: YB, CT, SL, JCC, and UW. Writing the first draft: YB. Writing with critical revision: YB, UW. All authors revised the secondary version and approved the final version of the manuscript.*

Ethical clearence

This study was performed in line with the principles of the Declaration of Helsinki. The Ethics Committee for medical and health sciences of the University of Khartoum, Sudan granted approval.

Informed concent

Written informed consent was obtained from the patients.

Concent for Publication

The authors affirm that human research participants provided informed consent for the publication of the images in Fig. 1.

Conflict of Interest

The authors declare no conflict of interest.

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[1] C. Blauwendraat, M.A. Nalls, A.B. Singleton, The genetic architecture of Parkinson's disease, Lancet Neurol [Internet] 19 (2) (2020) 170–178, https://doi.org/10.1016/S1474-4422(19)30287-X. Available from:.
[2] B.R. Bloem, M.S. Okun, C. Klein, Parkinson's disease, Lancet [Internet] 397 (10291) (2021) 2284–2303, https://doi.org/10.1016/S0140-6736(21)00218-X. Available from:.
[3] L. Ishihara-Paul, M.M. Hulihan, J. Kachergus, R. Upmanyu, L. Warren, R. Amouri, Mutations and parkinsonism, Neurolo 71 (12) (2008. Sep 16) 896–902. Sep 16.
[4] M.A. Chishti, S. Bohlega, M. Ahmed, A. Loualich, P. Carroll, C. Sato, et al., T313M PINK1 mutation in an extended highly consanguineous Saudi family with early-onset Parkinson disease, Arch. Neurol. 63 (10) (2006) 1483–1485.
[5] R.J. Keyser, S. Lesage, A. Brice, J. Carr, S. Bardien, Assessing the prevalence of PINK1 genetic variants in South African patients diagnosed with early- and late-onset Parkinson's disease, Biochem Biophys Res Commun [Internet] 398 (1) (2010) 125–129, https://doi.org/10.1016/j.bbrc.2010.06.049. Available from:.
[6] A.L. Leutenegger, M.A.M. Salih, P. Ib nez, M.M. Mukhtar, S. Lesage, A. Arabi, et al., Juvenile-onset parkinsonism as a result of the first mutation in the adenosine triphosphate orientation domain of PINK1, Arch. Neurol. 63 (9) (2006) 1257–1261.
[7] C. Cazeneuve, C. S
A new complex homozygous large rearrangement of the PINK1 gene in a Sudanese family with early onset Parkinson's disease, Neurogenetics 10 (3) (2009) 265–270.
[8] V. Bonifati, G. Rohe, E. Breedveld, E. Fabrizio, M. De Mari, C. Tassorelli, Early-onset parkinsonism associated with PINK1 mutations: frequency, genotypes, and phenotypes [1], Neurology 66 (7) (2006) 1129.
[9] S. Lesage, A. Lunati, M. Houot, S Ben Romdhan, F. Clot, C. Tesson, et al., Characterization of recessive Parkinson disease in a large multicenter study, Ann. Neurol. 88 (4) (2020) 843–850.
[10] M.E. Ibrahim, Genetic diversity of the Sudanese: insights on origin and implications for health, Hum. Mol. Genet. 30 (R1) (2021) R37–R41.
[11] Y. Bakhit, I. Schmitt, A. Hamed, E.A.A. Ibrahim, I.N. Mohamed, S.M. El-Sadig, et al., Methylation of alpha-synuclein in a Sudanese cohort, Park Relat Disord [Internet] 101 (June) (2022) 6–8, https://doi.org/10.1016/j.parkreldis.2022.05.009. Available from:.

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RESEARCH ARTICLE

PLA2G6-associated late-onset parkinsonism in a Sudanese family

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Abstract

Introduction: The phospholipase A2 group VI gene (*PLA2G6*) encodes an enzyme that catalyzes the hydrolytic release of fatty acids from phospholipids. Four neurological disorders with infantile, juvenile, or early adult-onset are associated with *PLA2G6* genetic alterations, namely infantile neuroaxonal dystrophy (INAD), atypical neuroaxonal dystrophy (ANAD), dystonia-parkinsonism (DP), and autosomal recessive early-onset parkinsonism (AREP). Few studies in Africa reported *PLA2G6*-associated disorders and none with parkinsonism of late adult onset. **Material and Methods**: The patients were clinically assessed following UK Brain Bank diagnostic criteria and International Parkinson and Movement Disorder Society's Unified Parkinson's Disease Rating Scale (MDS-UPDRS). Brain MRI without contrast was performed. Genetic testing was done using a custom-made Twist panel, screening 34 known genes, 27 risk factors, and 8 candidate genes associated with parkinsonism. Filtered variants were PCR-amplified and validated using Sanger sequencing and also tested in additional family members to study their segregation. **Result**: Two siblings born to consanguineous parents developed parkinsonism at the age of 58 and 60 years, respectively. MRI showed an enlarged right hippocampus in patient 2, but no overt abnormalities indicative of INAD or iron deposits. We found two heterozygous variants in *PLA2G6*, an in-frame deletion NM_003560: c.2070_2072del (p.Val691del) and a missense variant NM_003560:c.956C (p.Thr319Met). Both variants were classified as pathogenic. **Conclusion**: This is the first case in which *PLA2G6* is associated with late-onset parkinsonism. Functional analysis is needed to confirm the dual effect of both variants on the structure and function of iPLA2

Introduction

The Phospholipase A2 group VI gene (*PLA2G6* encodes an enzyme that catalyzes the hydrolytic release of fatty acids from phospholipids, producing lysophospholipids, which are used in deacetylation/reacetylation reactions. It possesses a calcium-dependent phospholipase activity, which has a role in phospholipid remodeling, an attribute consistent with cellular and mitochondrial membrane integrity and signal transduction.[1] It is expressed in the

brain and the gene product, iPLA2- nerve terminals and neuronal dendrites.[2] It has been demonstrated that decreased levels of iPLA2- disrupt the skeletal structure of the Golgi complex,[3] trafficking from the Golgi complex to the endoplasmic reticulum,[4,5] and impair Vps26 and Vps35 levels, retromer functions and ceramides levels in Parkinson's Disease (PD) models, similar to [2]

According to the age of onset and clinical features, PLA2G6-associated neurodegeneration (PLAN) can be categorized into 4 phenotypes: infantile neuroaxonal dystrophy (INAD), atypical neuroaxonal dystrophy (ANAD), adult-onset dystonia-parkinsonism (DP), and autosomal recessive early-onset parkinsonism (AREP). Symptoms of INAD and ANAD appear in infancy to early childhood and manifest mainly as progressive psychomotor deterioration, ataxia, spasticity, and axial dystonia.[6,7] Cerebellar atrophy and iron deposition in the basal ganglia are common radiological signs which are detected in most cases using magnetic resonance imaging (MRI) Therefore, PLAN could be classified as Neurodegeneration with Brain Iron Accumulation (NBIA).[8,9]

DP usually occurs between 20 and 40 years of age and manifests with parkinsonism, dystonia, and sometimes epilepsy. Earlier presentations in the second decade of life are also documented. In addition, impaired cognitive functions are common phenomena, and iron deposition incidence is scant.[10,11] The disease has a rapid progression and responds well to levodopa and dopamine receptor agonists and early-onset dyskinesia is common in patients who receive levodopa.[12]

In the early stages of their disease, PLA2G6-associated AREP patients exhibit bradykinesia, lower limb tremor, hypomimia, and gait disturbance. With the progression of the symptoms, additional features are observed in some cases, such as dystonia, dysarthria, dysphagia, cognitive impairment, and extrapyramidal signs and symptoms. Non-parkinsonian features may develop at later stages and may encompass cerebellar ataxia and autonomic dysfunction. Similar to DP, iron accumulation in the brain is rare. Instead, frontal and generalized white matter atrophy may be observed.[13,14] Moreover, a recent comprehensive article revealed that in some cases, mild to moderated cerebral and cerebellar atrophy was observed with a degree of variability in several newly identified cases.[15] Limb tremor and gai disturbance have favorable responses to dopaminergic agents.

To the best of our knowledge, no cases of adult-onset PLA2G6- associated parkinsonism have been reported in Africa. Here, we introduce the first case of genetically confirmed autosomal recessive parkinsonism with a late-onset disease in the sixth and seventh decades of life, identified in two siblings from a Sudanese family.

Material and Methods

Patients

A clinical diagnosis of PD was made in 2 patients using the United Kingdom Parkinson's Disease Society Brain Bank criteria[16] and confirmed using the International Parkinson and Movement Disorder Society's Unified Parkinson's Disease Rating Scale (MDS-UPDRS).[17] T1- and T2-weighted MRI brain scans were obtained for both patients without intravenous contrast. No T2 or SWI scans were performed.

The Research Ethics committee for Medical and Health studies at the Faculty of Medicine, University of Khartoum, Sudan, approved the study. Written informed consent was obtained from all participants.

Sampling

Five individuals (two patients, a healthy mother, and two healthy siblings) donated saliva for DNA extraction. One to two milliliters of saliva were collected using Oragene Discover. DNA purification was carried out following prepIT L2P manual protocol. DNA quality and quantity were performed using agarose gel electrophoresis and spectrophotometry (Nanodrop , Thermoscientific).

Genetic testing

We utilized Twist custom panel, which allows screening of 34 genes, 27 risk variants, and 8 candidate genes, which are mostly associated with Parkinsonism (panel details are available in the Table S1). The custom Twist EF Library Prep (Twist) was used to capture all exons, intron exon boundaries, 5 - and 3 -UTR sequences and 10-bp flanking sequences of target genes (RefSeq database, hg38 assembly). Specific probes for NGS target enrichment were designed using Twist software, and amplicon length varied between 250 and 500 bp. Runs were performed on Illumina MiSeq sequencer. The assay was performed according to the manufacturer's recommended protocol. Variants were prioritized based on the following criteria: frequencies
GnomAD) and our in-house database of 500 exomes, nucleotide and amino-acid conservation (based on alignments), relation of the gene to disease (per family), and inheritance pattern conservation (based on alignments).

Bioinformatics analysis of gene panel data

Human reference genome UCSC hg19 was used for sequence alignment and variant calling with the Burrows-Wheeler Aligner (BWA)[18] and the Genome Analysis Toolkit (GATK).[19] PCR duplicates were removed prior to variant

calling using Picard software. The mean coverage was 993 (range 594
was 98.7% (range 96.5
iants were annotated with ANNOVAR software.[20] Variants were prioritized based on the following criteria: frequencies

ents), relation of the gene to disease (per family), and inheritance pattern. We visualized the molecular effect of the missense variant on the structure of iPLA2- protein using UCSFC ChimeraX.[21]

Sanger sequencing

Filtered variants were PCR-amplified and validated (Sanger sequencing) to confirm the genotypes in affected individuals. The variants were Sanger-sequenced in three unaffected relatives (the mother, a non-identical twin to patient 1 and, a brother) to study the segregation. Forward and reverse primers were designed using Primer3 online tool.[22] Multiple sequence alignment was performed using Bioedit software v7.2.5[23] using default parameters.

Results

Clinical presentation

Two siblings born to consanguineous parents (first-degree cousins; Fig. 1) were diagnosed with PD. In the first patient (patient 1), symptoms appeared at the age of 58 years and started as a resting tremor in his right arm. Within three years, his condition developed into bilateral resting tremor in the upper limbs, with generalized slowness of movement and excessive salivation but with no drooling. Upon assessment, rigidity was detected with activation maneuvers in the neck and all limbs equally. Posture was not quite erect, and a mild disturbance of gait was detected. Symptoms improved with intake of levodopa with only minimal motor fluctuations. No dyskinesia was observed.

The second patient (patient 2) developed the first symptom at the age of 60 years in the form of resting tremor at her left arm. Her condition worsened within five years as the tremor markedly interfered with most of the daily activities, in addition to developing intermittent freezing of gait. Symptoms initially improved with levodopa however, at the time of examination the patient suffered from marked end-of-dose motor fluctuations and Levodopa-induced dyskinesia. This patient also reported frequen hallucinations sleeping problems (mainly insomnia), and inability to control urination. Upon assessment, she had hypomimia (i.e., facial masking), dysarthria and showed symptoms of dopamine dysregulation syndrome.

Upon assessment "on" levodopa, mild bradykinesia was observed in patient 1 while more progressive bradykinesia was observed in patient 2; neither of the two patients showed resting tremor while moderate rigidity

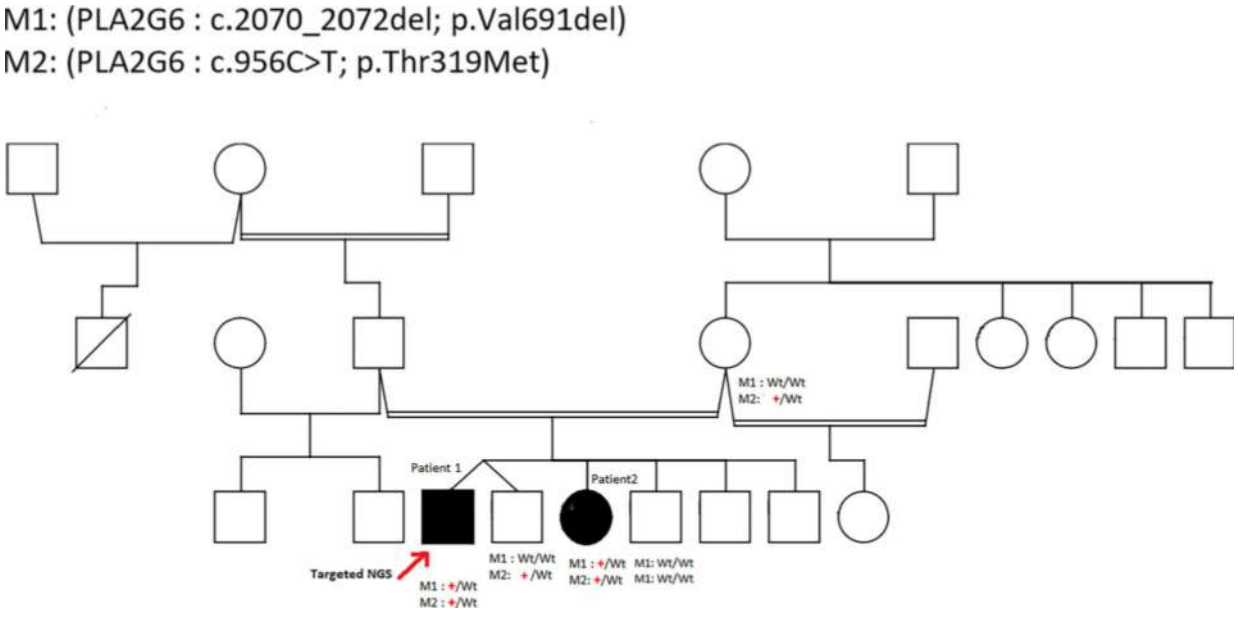

Figure 1. Heredogram showing the segregation result of two siblings diagnosed with autosomal recessive late onset Parkinsonism, their mother and two healthy sibling . the first variant (p.Val691del) was considered M1 while the second variant (p.Thr319Met) is considered M2. The red plus indicates a carrier of the variant while the wild type is indicated with (wt). The red arrow indicated the targeted individual for the panel.

in both patients was found in all extremities and neck. We were unable to perform a formal detailed cognitive assessment.

Investigations and radiographic finding

We performed fundoscopy, lipid profile, high-resolution lipoprotein electrophoresis, peripheral blood smear, serum iron, serum ferritin, and transferrin saturation studies for both patients. Prompted by a history of hearing impairment in patient 2, we performed Ear, Nose, and Throat examination as well as audiometric testing. All these investigations were unremarkable for both patients. We found moderate hippocampal expansion on T2-weighted MRI brain scans of patient 1 (Fig. 2). Brain MRI was done without intravenous contrast and did not included T2 nor SWI sequences.

Identified variants using the genetic panel

Two heterozygous variants in *PLA2G6* (NM_003560.4: c.2070_2072de (p.Val691del an NM_003560.4: c.956C

S2. We were also able to analyze copy number variations from the panel which was unremarkable for both patients.

Analysis of variants

Both variants found in *PLA2G6* were validated using Sanger sequencing. Segregation analysis (Fig. 1) showed that the mother and one healthy sibling were carriers for one variant (p.Thr391Met). A sample from the father who was deceased at the time of sampling
able. Thus, confirming the paternal origin of the other variant (p.Val691del) was not feasible. The mother and two healthy siblings had reference alleles at the genomic location tested (wildtype). Chromatograms of all family members tested are available in Fig. S1. Although a Trans-configuration of the two variants in the patients could not be verified with certainty (due to the use of short read sequencing and the lack of a paternal sample), this segregation pattern is highly suggestive of an autosomal recessive inheritance in a compound heterozygous state. The variants were classified as pathogenic in these patients based on the ACMG criteria given in Supplementary 3. As predicted in Fig. 3, the mutant residue will inflict at least 4 clashing points with neighboring residues, which may affect the structure, and hence the function of the protein.

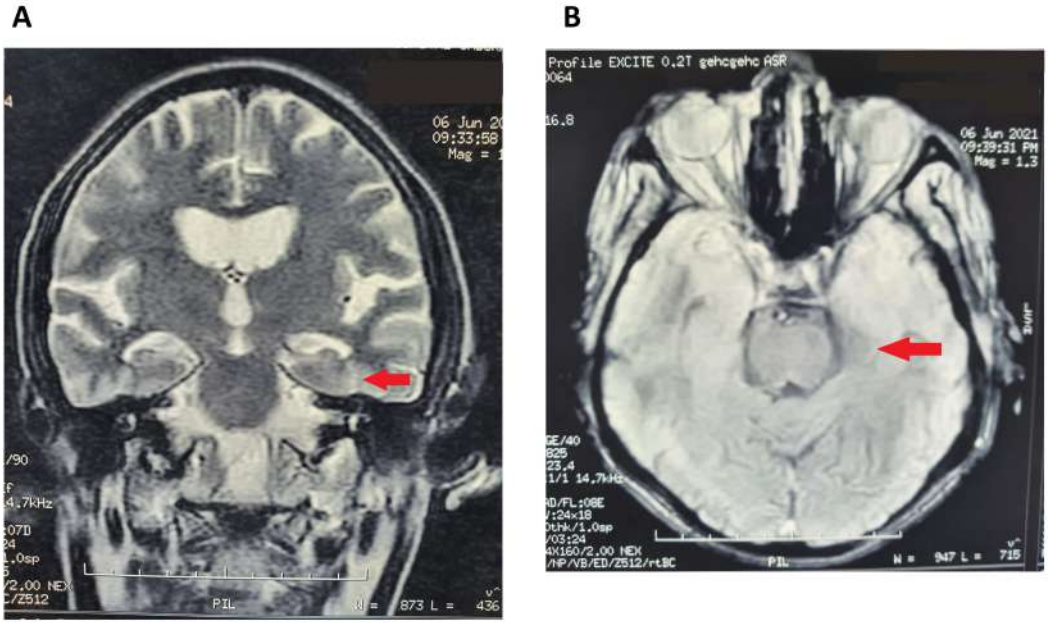

Figure 2. T2-weighted MRI brain scans of patient 1 showing moderate hippocampal expansion on the left side, as indicated with the red arrow. (A) Coronal section. (B) Horizontal section.

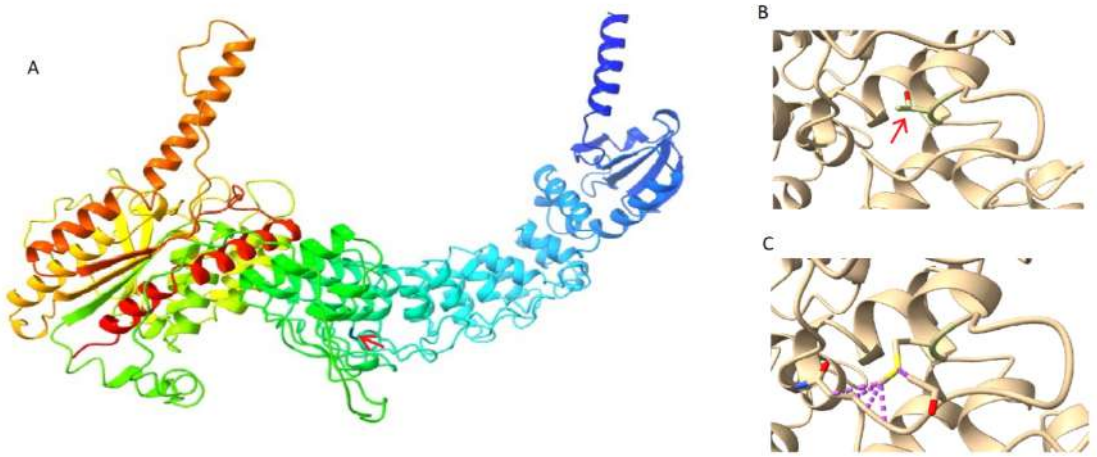

Figure 3. Homology modeling of iPLA2- showing the effect of the mutant variant (p.Thr319Met)on the structure of the protein, inflicting at least four clash point (indicated with dotted purple lines) with the surrounding amino acid residues. (A) Three-dimensional structure of the protein iPLA2-

Discussion

In a consanguineous Sudanese family, we identified two variants in *PLA2G6* in a compound heterozygous state in two patients with late onset parkinsonism. Pathogenic variants in this gene have been associated with autosomal recessive juvenile onset or early-onset parkinsonism (OMIM #612953; PARK14). To our knowledge, this is the first case in which a *PLA2G6* variant is associated with late-onset autosomal recessive presentation. Yet, it is still consistent with the complex nature of *PLA2G6- associated* phenotypes and its tendency to feature multiple neurological disorders despite the rarity of its mutations.

The first variant we identified is a known pathogenic in-fram deletion (NM_003560.4:c.2070_207 del; p.Val691del) which, unlike our study, was reported in a homozygous state in all previous studies, and all reported individuals were cases of INAD.[24,25] *PLA2G6 associated* INAD was also reported in another Sudanese family, where a homozygous pathogenic variant affecting a canonica splice site (NM_003560.2:c.1427 found in the two affected siblings.[26]

The second variant is also a known missense variant (NM_003560.4:c.956C

[15] A previous functional evaluation performed on fibroblasts from a patient carrying this variant

[27]

Apart from the unusually late age of onset, the presentation of the disease was consistent with the phenotypes of previous patients carrying p.Val691del or p.Thr319Met, and also with what is known about adult-onset *PLA2G6* related parkinsonism in *general. PLA2G6- associated* hippocampal changes have been previously described in number of previous studies.[28] In this study, MRI of one proband but not the other showed hippocampal expansion. Hippocampal involvement was also described by Michelis and colleagues, reporting a novel *PLA2G6* mutation in a 35-year-old patient with adult-onset autosomal recessive parkinsonism and early stages of dementia.[29] In contrast, neither of our patients showed signs of dementia.

This study had limitations Although one of our patients had a history of psychiatric manifestations, it was not feasible to perform a formal, detailed cognitive and psychiatric assessment. Also, iron deposition in the brain cannot be reliably ruled out as T2 and SWI MRI sequences were not obtained. Last, using a panel limits the chance of detecting any other possible molecular findings (e.g., intronic variants or coding variants in other genes not included in the panel) which are classically detectable with WES or WGS.

Conclusion

We identified two *PLA2G6* variants in two patients from a consanguineous Sudanese family, consistent with a genetic diagnosis of autosomal recessive parkinsonism. Although both variants were previously reported, these patients had an unusually late onset of disease in the sixth decade of life, expanding the age range of the *PLA2G6*-associated parkinsonism. Functional analysis of the effects

of these variants on the structure and function of iPLA2 may unravel the mechanisms of this variability.

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Authors Contribution

Conception and design: UW, YB, CT, J-C C, SL, OS, LEOE; Primary clinical evaluation: OS and YB. Secondary clinical assessment (UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria AND MDS-UPDRS), participant recruitment and sampling: YB, MI, KE, and IE. DNA preparation and PCR: YB: Target NGS analysis: CT. Data analysis and interpretation: YB, CT, J-C C, SL and UW. Writing the first draft: YB. Writing with critical revision: YB, UW, J-C C, and LEOE. All authors revised and approved the final version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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References

1. Larsson Forsell PKA, Kennedy BP, Claesson HE. The human calcium-independent phospholipase A2 gene: multiple enzymes with distinct properties from a single gene. Eur J Biochem. 1999;262(2):575-585. doi:10.1046/j.1432-1327.1999.00418.x
2. Lin G, Lee PT, Chen K, et al. Phospholipase *PLA2G6*, a parkinsonism-associated gene, affects Vps26 and Vps35, Retromer function, and ceramide levels, similar to synuclein gain. Cell Metab. 2018;28(4):605-618.e6. doi:10.1016/j.cmet.2018.05.019
3. De Figueiredo P, Polizotto RS, Drecktrah D, Brown WJ. Membrane tubule-mediated reassembly and maintenance of the Golgi complex is disrupted by phospholipase A2 antagonists. Mol Biol Cell. 1999;10(6):1763-1782. doi:10.1091/mbc.10.6.1763
4. De Figueiredo P, Drecktrah D, Katzenellenbogen JA, Strang M, Brown WJ. Evidence that phospholipase A2 activity is required for Golgi complex and trans Golgi network membrane tubulation. Proc Natl Acad Sci U S A. 1998;95(15):8642-8647. doi:10.1073/pnas.95.15.8642
5. De Figueiredo P, Drecktrah D, Polizotto RS, Cole NB, Lippincott-Schwartz J, Brown WJ. Phospholipase A2 antagonists inhibit constitutive retrograde membrane traffic to the reticulum. Traffic. 2000;1(6):504-511. doi:10.1034/j.1600-0854.2000.010608.x
6. Seitelberger F. Neuropathological conditions related to neuroaxonal dystrophy. Acta Neuropathol. 1971;5:17-18. doi:10.1007/978-3-642-47449-1_3
7. Riku Y, Ikeuchi T, Yoshino H, et al. Extensive aggregation of
dystrophy: an autopsied individual with a novel mutation in the *PLA2G6* gene-splicing site. Acta Neuropathol Commun. 2014;2(1):1-11. doi:10.1186/2051-5960-1-12
8. Schneider SA, Hardy J, Bhatia KP. Syndromes of neurodegeneration with brain iron accumulation (NBIA): an update on clinical presentations, histological and genetic underpinnings, and treatment considerations. Mov Disord. 2012;27(1):42-53. doi:10.1002/mds.23971
9. Hayflick SJ, Kurian MA, Hogarth P. Neurodegeneration with brain iron accumulation. Handb Clin Neurol. 2018;147:293-305. doi:10.1016/B978-0-444-63233-3.00019-1
10. Paisán-Ruiz C, Li A, Schneider SA, et al. Widespread Lewy body and tau accumulation in childhood and adult onset dystonia-parkinsonism cases with *PLA2G6* mutations. Neurobiol Aging. 2012;33(4):814-823. doi:10.1016/j.neurobiolaging.2010.05.009
11. Karkheiran S, Shahidi GA, Walker RH, et al. Case reports *PLA2G6*-associated Dystonia
and literature review. Tremor Other Hyperkinet Mov (N Y). 2015;5:317. doi:10.7916/D84Q7T4W
12. Paisán-Ruiz C, Guevara R, Federoff M, et al. Early-onset L-dopa-responsive parkinsonism with pyramidal signs due to ATP13A2, PLA2G6, FBXO7 and Spatacsin mutations. Mov Disord. 2010;25(12):1791-1800. doi:10.1002/mds.23221
13. Shi CH, Tang BS, Wang L, et al. *PLA2G6* gene mutation in autosomal recessive early-onset parkinsonism in a Chinese cohort. Neurology. 2011;77(1):75-81. doi:10.1212/WNL.0b013e318221acd3
14. Giri A, Guven G, Hanagasi H, et al. *PLA2G6* mutations related to distinct phenotypes: a new case with early-onset parkinsonism. Tremor Other Hyperkinet Mov (N Y). 2016;2016:1-6. doi:10.7916/D81G0M12
15. Magrinelli F, Mehta S, Di Lazzaro G, et al. Dissecting the phenotype and genotype of *PLA2G6*-related parkinsonism. Mov Disord. 2022;37(1):148-161. doi:10.1002/mds.28807
16. Daniel SE, Lees AJ. Parkinson's disease society brain Bank, London: overview and research. J Neural Transm Suppl. 1993;39:165-172.
17. Goetz CG, Tilley BC, Shaftman SR, et al. Movement Disorder Society-sponsored revision of the unified Parkinson's disease rating scale (MDS-UPDRS): scale

presentation and clinimetric testing results. Mov Disord. 2008;23(15):2129-2170. doi:10.1002/mds.22340
18. Li H, Durbin R. Fast and accurate long-read alignment with burrows-wheeler transform. Bioinformatics. 2010;26(5):589-595. doi:10.1093/bioinformatics/btp698
19. McKenna A, Hanna M, Banks E, et al. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20(9):1297-1303. doi:10.1101/gr.107524.110
20. Mitsui J, Takahashi Y, Goto J, et al. Mechanisms of genomic instabilities underlying two common fragile-site-associated loci, PARK2 and DMD, in germ cell and cancer cell lines. Am J Hum Genet. 2010;87(1):75-89. doi:10.1016/j.ajhg.2010.06.006
21. Pettersen EF, Goddard TD, Huang CC, et al. UCSF ChimeraX: structure visualization for researchers, educators, and developers. Protein Sci. 2021;30(1):70-82. doi:10.1002/pro.3943
22. Untergasser A, Cutcutache I, Koressaar T, et al. Primer3-new capabilities and interfaces. Nucleic Acids Res. 2012;40(15):1-12. doi:10.1093/nar/gks596
23. Hall TA. BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95-98.
24. Khateeb S, Flusser H, Shelef I, et al. *PLA2G6* mutation underlies infantile neuroaxonal dystrophy. Am J Hum Genet. 2006;79(November):942-948.
25. Kuang G, O'Brien S, Thomas D, et al. public access. Bone. 2014;23(1):1-7. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf
26. Elsayed LEO, Mohammed IN, Hamed AAA, et al. Case report of a novel homozygous splice site mutation in *PLA2G6* gene causing infantile neuroaxonal dystrophy in a Sudanese family. BMC Med Genet. 2018;19(1):1-5. doi:10.1186/s12881-018-0592-y
27. Drecourt A, Babdor J, Dussiot M, et al. Impaired transferrin receptor Palmitoylation and recycling in neurodegeneration with brain iron accumulation. Am J Hum Genet. 2018;102(2):266-277. doi:10.1016/j.ajhg.2018.01.003
28. Balicza P, Bencsik R, Lengyel A, et al. Novel dominant MPAN family with a complex genetic architecture as a basis for phenotypic variability. Neurol Genet. 2020;6(5):e515. doi:10.1212/NXG.0000000000000515
29. Michelis JP, Hattingen E, Gaertner FC, et al. Expanded phenotype and hippocampal involvement in a novel compound heterozygosity of adult *PLA2G6* associated neurodegeneration (PARK14). Park Relat Disord. 2017;37:111-113. doi:10.1016/j.parkreldis.2017.01.005

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1.
Appendix S2.
Appendix S3.
Figure S1.

3.4 Publication 4: (in progress)

Novel in-frame variant in CPS1 is associated with autosomal recessive Parkinsonism

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Abstract:
Parkinson's disease and other neurodegenerative disorders are characterized by the progressive degeneration of neurons in the central nervous system. While the exact etiology of these disorders is multifaceted and not fully understood, emerging evidence suggests that metabolic dysregulation may play a role in their pathogenesis. One enzyme that has garnered attention in the context of neurodegenerative diseases is carbamoyl phosphate synthase 1 (CPS1). CPS1 is primarily known as the rate-limiting enzyme in the urea cycle, responsible for the detoxification of ammonia in the liver. It catalyzes the conversion of ammonia and bicarbonate into carbamoyl phosphate, a critical step in ammonia detoxification throughout the body. In our study we collected saliva samples from five Parkinson's disease patients for DNA extraction. Targeted next-generation sequencing (NGS) was performed using a Twist custom panel capable of screening 34 genes, 27 risk variants, and 8 candidate genes associated with Parkinsonism. Additionally, whole exome sequencing was conducted using the Illumina NovaSeq 6000 sequencer. Our findings revealed a novel in-frame insertion, specifically NM_001122633:c.15_16insTTC, which was segregating as homozygous in three families and in compound heterozygous in two families with the novel double missense variant NM_001875c.1030_1032GCT to segregate in all families studied. However, further investigation is necessary to determine the precise cellular and molecular effects of this variant and to confirm its significance in relation to Parkinson's disease. Understanding the impact of CPS1 and its variants on neurodegenerative disorders like Parkinson's disease is crucial for elucidating the underlying mechanisms and developing targeted therapies. Further studies are warranted to validate our findings and explore the functional implications of this variant in the context of Parkinson's disease pathogenesis.

Introduction
Parkinson's disease and other neurodegenerative disorders are characterized by the progressive degeneration of neurons in the central nervous system. While the exact etiology of these disorders is multifaceted and not fully understood, emerging evidence suggests that metabolic dysregulation may play a role in their pathogenesis. Carbamoyl phosphate synthase 1 (CPS1) is an enzyme located in the mitochondrial matrix which is considered the rate limiting enzyme role in the urea cycle, which is responsible for the detoxification of ammonia in the liver. It catalyzes the conversion of ammonia and bicarbonate into carbamoyl phosphate, a crucial step in the detoxification of ammonia in the body (1). However, recent research suggests that CPS1 may have additional functions beyond the urea cycle, including roles in cellular metabolism and neurodegenerative processes.
The involvement of CPS1 in neurodegenerative disorders extends beyond Parkinson's disease. Two novel missense variant were detected in CPS1 in patients diagnosed with hyperammonemia and leukodystrophy (2), In another study altered CPS1 activity was observed in brain tissues of individuals with Alzheimer's disease(3). Additionally a study by Silvestri et al. (2017) reported dysregulation of CPS1 in the striatum, cortex, and cerebellum of Huntington's disease patients. These findings suggest a potential connection between CPS1-related metabolic dysregulation and neurodegeneration in various disorders (4).
Understanding the role of CPS1 in neurodegenerative disorders opens avenues for potential therapeutic interventions. By targeting CPS1 activity and associated metabolic

pathways, it may be possible to modulate disease progression. Future research could explore the development of small molecules or gene therapy approaches aimed at restoring CPS1 function, reducing ammonia toxicity and improving neuronal health.

Materials and methods:

Ethics: The Research Ethics Committee for Medical and Health Studies at the Faculty of Medicine, University of Khartoum approved this study Informed consent was obtained from all participants.

Patients Characterization and Clinical Diagnosis: The clinical diagnosis of Parkinson's disease (PD) was conducted based on the United Kingdom Parkinson's Disease Society Brain Bank criteria (5), and secondary Parkinsonism was excluded. Additionally the family histories of the participants were negative for Essential Tremor and Alzheimer's disease. The Movement Disorders Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) was used to characterize PD patients (6). Video recordings of the clinical assessment were saved for future reference. All assessments were conducted in the morning, with patients refraining from taking antiparkinson medications for 12-14 hours.

Sampling and Genetic Testing: Sampling: eleven individuals from five consanguineous, multi-generational Sudanese families provided saliva samples for DNA extraction. The samples consisted of five patients and six symptom-free siblings. Oragene Discover® collection kits were used for saliva collection. DNA purification followed the prepIT® L2P manual protocol. DNA quality and quantity were assessed using agarose gel electrophoresis and spectrophotometry (Nanodrop®, Thermoscientific).

Genetic Testing: Target Panel Next Generation Sequencing: The study employed a Twist custom panel capable of screening 34 genes, 27 risk variants, and 8 candidate genes associated with Parkinsonism. The custom Twist EF Library Prep (Twist) was used to capture all exons, intron-exon boundaries, 5'- and 3'-UTR sequences, and 10-bp flanking sequences of the target genes based on the RefSeq database (hg38 assembly). Twist software was used to design specific probes for NGS target enrichment, with amplicon length ranging from 250 to 500 bp. Illumina MiSeq sequencer was used for the sequencing runs, following the manufacturer's recommended protocol. Variant prioritization was based on several criteria, including frequencies below 0.01% in public databases (ExAC/GnomAD) and an in-house database of 500 exomes, conservation at the nucleotide and amino acid levels, gene-disease relation (per family), and inheritance pattern conservation.

Bioinformatics Analysis of Gene Panel Data: The Burrows-Wheeler Aligner (BWA)(7) and the Genome Analysis Toolkit (GATK) (8) were utilized for sequence alignment and variant calling, using the human reference genome UCSC hg38. PCR duplicates were removed prior to variant calling with Picard software. Variant annotation was performed using ANNOVAR software. The mean coverage for all individuals tested was 993x (range: 594-1241x), with an average percent coverage at 30x of 98.7% (range: 96.5-99.6%).

Whole Exome Sequencing:

DNA from 11 individuals representing 5 families, who were unsolved cases from the targeted panel NGS, were subjected to whole exome sequencing (WES).

Library Preparation: For the enrichment of the exome, we utilized the Human Core Exome kit in combination with the RefSeq Panel kit from Twist Bioscience (San Francisco, USA). The capturing of all exonic regions is described in the following link: [Link: Human Core Exome RefSeq Panel Bed Files].

The whole exome sequencing was conducted at the University Hospital Bonn NGS core facility using the Illumina NovaSeq 6000 sequencer (San Diego, California, United States). This platform generates a median coverage of 100x with paired-end reads of 100 base pairs: https://wwwtwistbioscience.com/resources/data-files/human-core exomerefseq-panel-bed-files.

Bioinformatics Analysis: The resulting high-quality paired reads were aligned to the human reference genome hg38 using the Burrows-Wheeler Aligner (7)(BWA-MEM) algorithm v0.7.12 with default parameters. The resulting BAM files were processed to remove any PCR duplicates, and sorting and indexing were performed using samtools v1.7 (9) .

Variant calling was carried out using Freebayes v1.0.0 (10) with default parameters, and the resulting variant call format (VCF) files were filtered based on depth (>20) and genotype quality (>20). The processed VCF files were annotated and prioritized using VarAFT annotation and filtering tools. Variants were filtered based on a minor allele frequency cut-off of 0.01 in the GnomAD genome database, the variant's effect, and in-silico prediction pathogenicity tools.

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Initially focus was placed on variants with major structural effects. Subsequently attention was directed toward missense variants annotated as pathogenic by SIFT and PolyPhen software, along with in-frame variants. Genes associated with the Online Mendelian Inheritance in Man (OMIM) disease-related genes and the Orphanet database were checked.

The resulting variant status was evaluated following the guidelines of the American College of Medical Genetics and Genomics (ACMG, Bethesda, United States). Variants of unknown significance, likely pathogenic, or pathogenic, confirmed using Variant Effect Predictor (VEP), available at [Link: Ensembl VEP], were further analyzed by amplifying and sequencing them using Sanger sequencing to confirm segregation.

Segregating variants were cross-referenced with the Sudanese exome, which consists of 131 exomes, to estimate allele frequency and identify possible Identical by Descent (IBD) sharing to rule out population polymorphisms.

Result:

Five patients from five unrelated families were diagnosed with autosomal recessive parkinsonism, three of them were with juvenile onset (PD021, PD033) while the remaining three were of an early onset (PD023, PD025 and PD030). The average age of onset was 29.4 +/- 15 years and the female/ male ratio was 0.25. Only bradykinesia and rigidity were prevalent in all affected individuals with a wide range of disease progression (table1).We found the novel likely pathogenic variant CPS1: NM_001122633:c.15_16insTTC, which was recurrent in homozygous state in PD021A, PD030A and PD033A and heterozygous in PD023A and PD025A. A second novel likely pathogenic variant CPS1: NM_001875c.1030_1032GCT was found heterozygous in both families PD023 and PD025, suggesting a compound heterozygous state, (Details of ACMG classifications are available in supplementary1. Using Sanger sequencing, we were able to confirm co-segregation all indexed family members (figure 1). Details of chromatograph of all indexed individuals are available in supplementary figure2. We also performed tandem repeat analysis for a gene panel that was designed and executed in house (German centre for neurodegenerative research DZNE- Bonn) to role out possible repeat expansions and they were of no significant relevance.

Discussion:

In the current study five patients from five unrelated families were diagnosed with autosomal recessive Parkinson's disease. Interestingly although patients demonstrate some individually unique features of the disease, they have some interesting common features of the disease; all patients have relatively mild to moderate parkinsonian features, resting tremor is absent from all patients while rigidity is prevalent in all of them. No cognitive manifestations were observed in all probands while all patients showed good response to L-dopa. Another interesting feature is that to of the three homozygous carriers of the variant have Juvenile onset of the condition while for the remaining patients it is adult onset parkinsonism. The age of onset- zygocity relation is not uncommon for Parkinson's disease, a typical example would be PLA2G6 associated Parkinsonism (11).

Recently studies have suggested that CPS1 may also have a role in neurodegenerative diseases such as Alzheimer and Parkinson's disease. One study by Shan et al. (2021) found that CPS1 was significantly upregulated in the brain tissue of Alzheimer's patients compared to healthy controls. They also found that increased CPS1 expression was associated with increased beta-amyloid protein levels, which is a hallmark of Alzheimer's disease(12).

In another study CPS1 expression was increased in the brains of mice with Parkinson's disease. They also found that treatment with a CPS1 inhibitor improved motor function and reduced neuroinflammation in these mice (13).

Similarly CPS1 expression was increased in the brains of mice with Huntington's disease. They also found that treatment with a CPS1 inhibitor improved motor function and reduced neurodegeneration in these mice (14).

These findings suggest that CPS1 may play a role in the pathogenesis of neurodegenerative diseases, and that targeting CPS1 may be a potential therapeutic strategy for these conditions. However, more research is needed to fully understand the mechanisms underlying the role of CPS1 in neurodegeneration and to develop effective treatments targeting this enzyme.

There is growing evidence that suggests a relationship between urea and Parkinson's disease (PD), a neurodegenerative disorder characterized by the loss of dopamine-producing neurons in the brain.

Urea levels were significantly elevated in the cerebrospinal fluid (CSF) of patients with PD compared to healthy controls. The authors suggest that elevated urea levels may be a result of impaired renal function in these patients (15). However, other studies have reported conflicting results. Urea levels were not significantly different between PD patients and healthy controls. The authors suggest that the differences in results between studies may be due to differences in the populations studied and the methods used to measure urea levels (16).

It is worth mentioning that while there are strong suggested evidence for the possible role of CPS1 in the pathogenesis of Parkinson's disease, no variant was attributed to PD case other than the current study the current finding could set up a vital starting point for further investigation to demonstrate evidence of what have already been addressed on cellular and molecular level.

Conclusion:

We found novel in frame insertion NM_001122633:c.15_16insTTC in five families and NM_001875c.1030_1032GCT in two of the five families, which are, diagnosed with autosomal recessive Parkinsonism, in the gene CPS1, which is consistent with the mode of inheritance of CPS1 related conditions, Overall, the relationship between urea

and PD is complex and requires further investigation. While some studies suggest that urea may play a role in the pathogenesis of PD and could be a potential therapeutic target, other studies have reported conflicting results. Further research is needed to fully understand the relationship between urea and PD and to determine whether targeting urea is a viable therapeutic strategy for this disease. These findings suggest that CPS1 may play a role in the pathogenesis of neurodegenerative diseases, and that targeting CPS1 may be a potential therapeutic strategy for these conditions. However, more research is needed to fully understand the mechanisms underlying the role of CPS1 in neurodegeneration and to develop effective treatments targeting this enzyme.

Reference:

1. Herman H, Shambaugh E. Shambaugh1977. 2018;(February):2083–7.
2. Chen X, Yuan L, Sun M, Liu Q, Wu Y. Two novel CPS1 mutations in a case of carbamoyl phosphate synthetase 1 deficiency causing hyperammonemia and leukodystrophy. J Clin Lab Anal. 2018;32(5):2–7.
3. Gregg EW, Shaw JE. Global Health Effects of Overweight and Obesity. N Engl J Med. 2017;377(1):80–1.
4. Silvestri L, et al. Carbamoyl phosphate synthetase 1 deficiency: A clinical, biochemical and molecular characterization. Clin Genet. 2017;91(5):780–5.
5. Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. Arch Neurol [Internet]. 1999 Jan [cited 2016 Oct 21];56(1):33–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9923759
6. Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, et al. Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Scale presentation and clinimetric testing results. Mov Disord. 2008;23(15):2129–70.
7. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 2010;26(5):589–95.
8. Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, del Angel G, Levy-Moonshine A, et al. From fastQ data to high-confidence variant calls: The genome analysis toolkit best practices pipeline. Current Protocols in Bioinformatics. 2013.
9. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25(16):2078–9.
10. Garrison E, Marth G. Haplotype-based variant detection from short-read sequencing. 2012;1–9. Available from: http://arxiv.org/abs/1207.3907
11. Magrinelli F, Mehta S, Di Lazzaro G, Latorre A, Edwards MJ, Balint B, et al. Dissecting the Phenotype and Genotype of PLA2G6-Related Parkinsonism. Mov Disord. 2022;37(1):148–61.
12. Shan C, et al. Increased CPS1 expression is associated with Alzheimer's

disease pathology Brain Res Bull. 2021;169:108–16.

13. Liu L et al. Inhibition of CPS1 ameliorates neuroinflammation and oxidative stress in Parkinson's disease. Biochem Biophys Res Commun. 2020;521(1):145–51.

14. Y C et al. Inhibition of carbamoyl phosphate synthase 1 as a therapeutic strategy in a mouse model of Huntington's disease. Exp Neurol. 2019;312:58–67.

15. W M et al. Urea Levels in Cerebrospinal Fluid and Parkinson's Disease. Front Aging Neurosci. 2018;10:207.

16. DF T et al. Cerebrospinal fluid markers of central urea cycle metabolism correlate with disease severity in Huntington's disease. J Huntingtons Dis. 2018;7(3):259–69.

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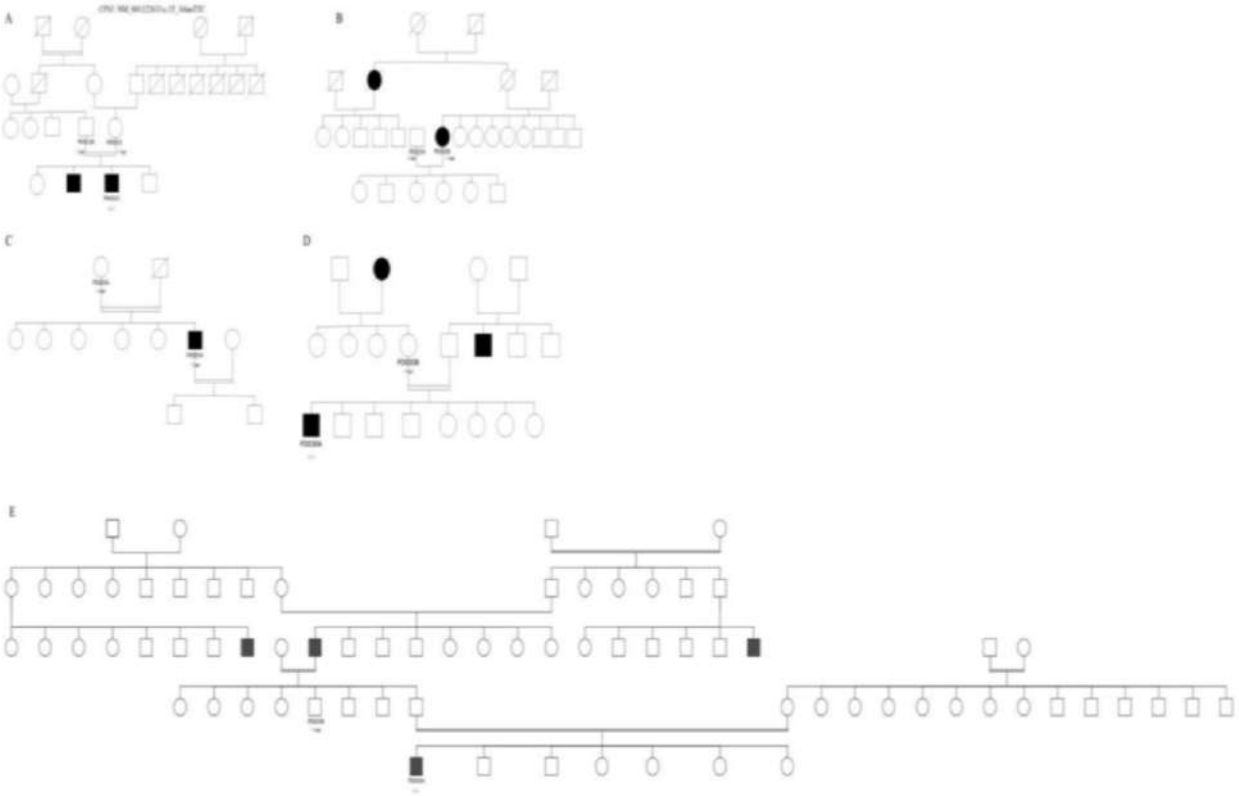


Figure 1: heredograms of showing the the variant NM_001122633:c.15_16insTTC zygocity state. Indexed individuals were identified as follow: PD0XX for the family number, and the letter next to the number is the particular individual in the family

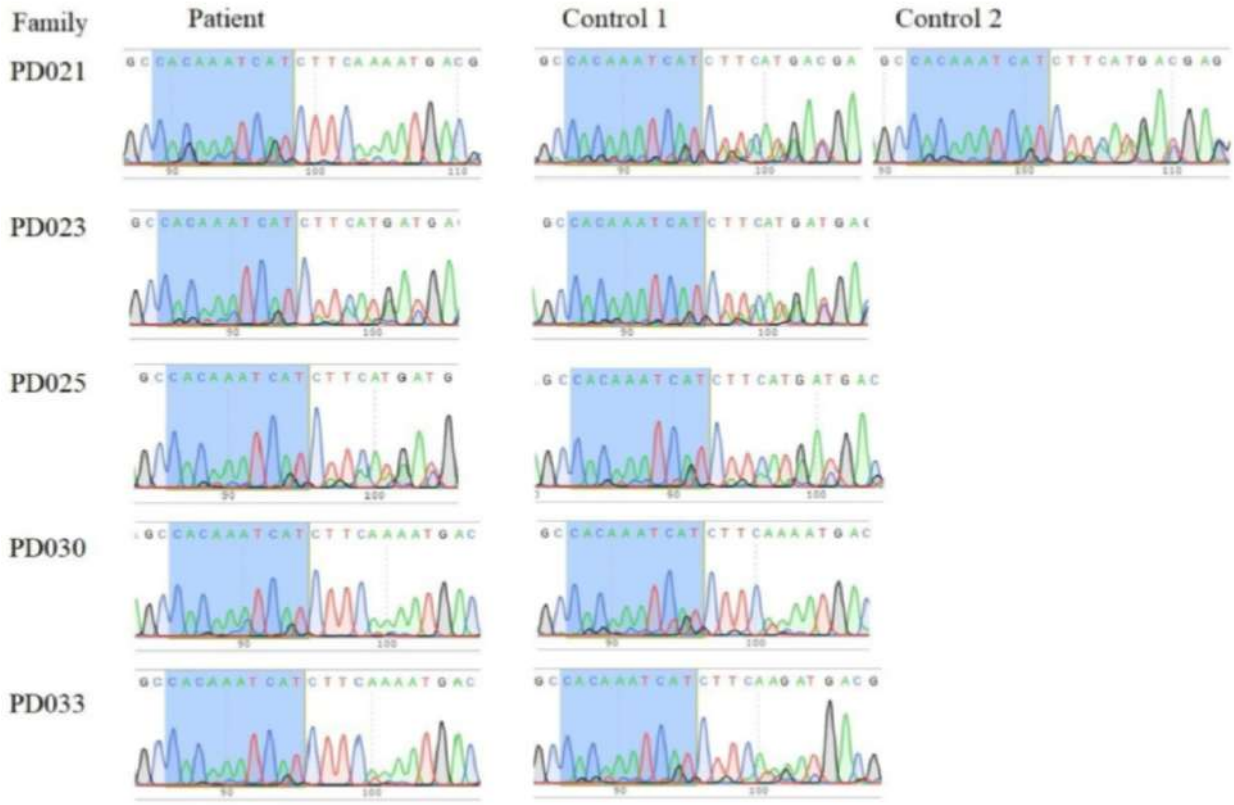
Table1: clinical feature of PD family cohort (retrieved from MDS-UPDRS patients records)

Patient's code	Sex	Age of onset (years)	Age at examination (years)	Bradykinesia (in the most affected limb) (0-4)	Postural instability (0-4)	Rigidity (in the most affected limb) (0-4)	Resting Tremor (in the most affected limb) (0-4)	Hoehn and Yahr stage (0-5)	Depression / Anxiety	Cognitive impairment
PD021A	M	7	18	2	1	1	0	2	No	No
PD023B	F	39	39	1	0	1	0	1	Yes	No
PD025A	M	40	70	2	1	2	0	2	No	No
PD030A	M	45	50	3	0	2	0	3	Yes	No
PD033A	M	16	16	1	1	1	0	1	No	No

Supplemetary table1:

Variant & classification	ACMG Evidence	Criteria	Description
CPS1: p.I5delinsIF Classification: likely pathogenic	PM4	Moderate	Protein coding length changes as a result of in frame variant, and this variant is not located in a repeat region.
	PM2	Supporting	Variant is not found in gnomAD
	PS3	Strong	Well established in vitro or in vivo functional studies supportive of the damaging effect of the gene or the gene's product.
	PP1	Supporting	Co-segregation with disease in multiple affected family members
	PP3	Supporting	Multible lines of computational evidence support

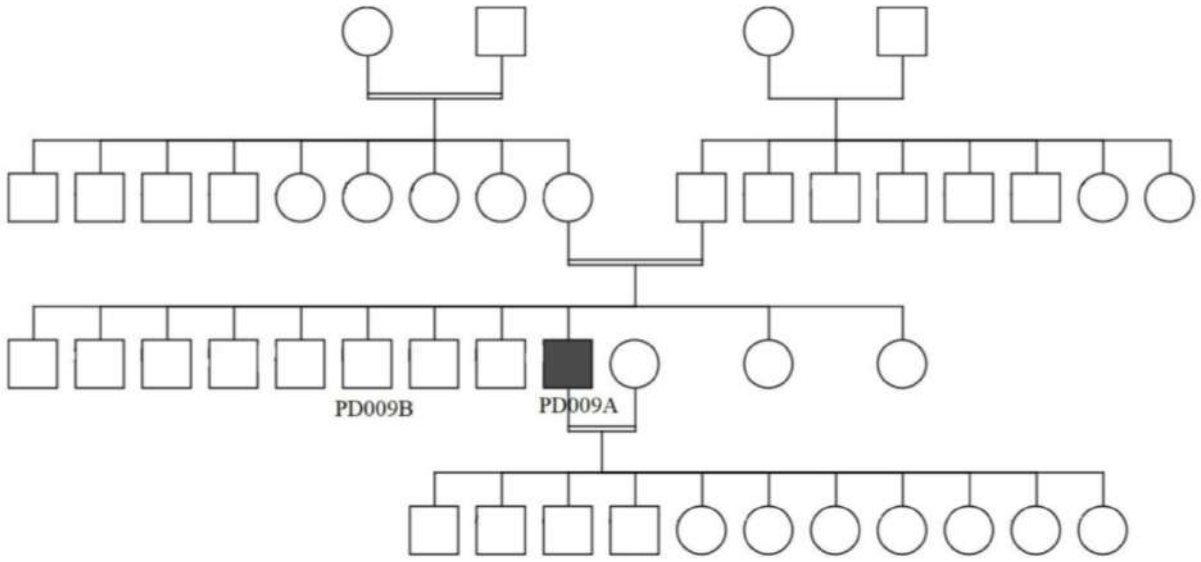
			deleterious effect of the gene or the gene's product
CPS1: p.T344A Classification: Likely pathogenic	PP4	Supporting	Patient's phenotype or family history is highly specific for monogenic disease
	PM2	Supporting	Variant not found in GnomAD and 1000 genome
	PM1	Moderate	Located in a mutational hot spot
	PM5	Moderate	Novel missense change at an amino acid residue where whee a different missense pathogenc change has been seen before

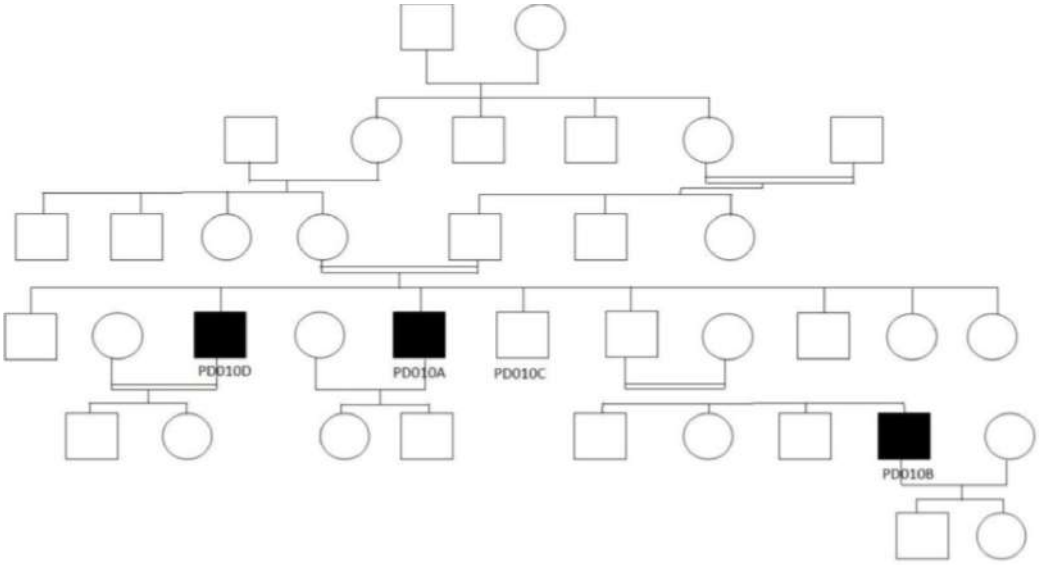


Sup fig 1 : chromatogram of all indexed individuals showing the zygocity state for the variant NM_001122633:c.15_16insTTC.

3.5 Non concluded families
3.5.1 Family pedigrees

Fifty patients from thirty families were diagnosed with autosomal recessive juvenile/early onset parkinsonism. The average age of onset is 37 +/- 24.7 and the female to male ratio is 0.28.

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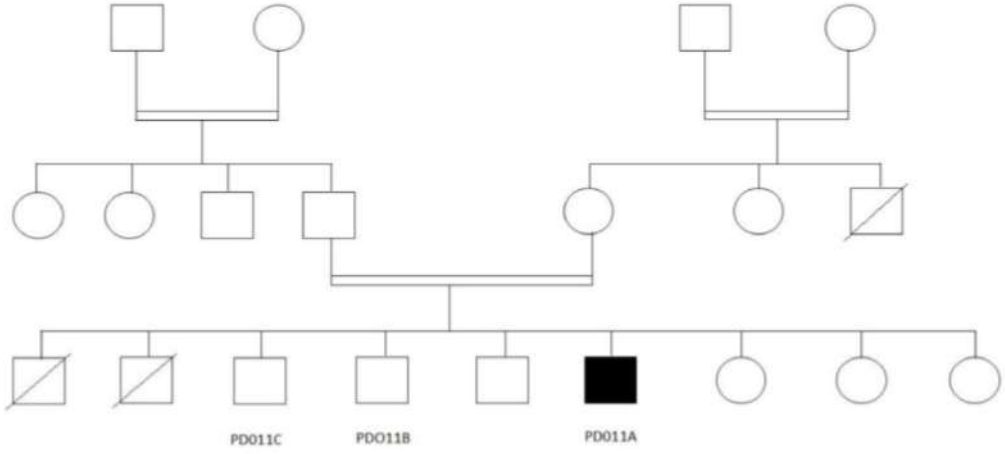
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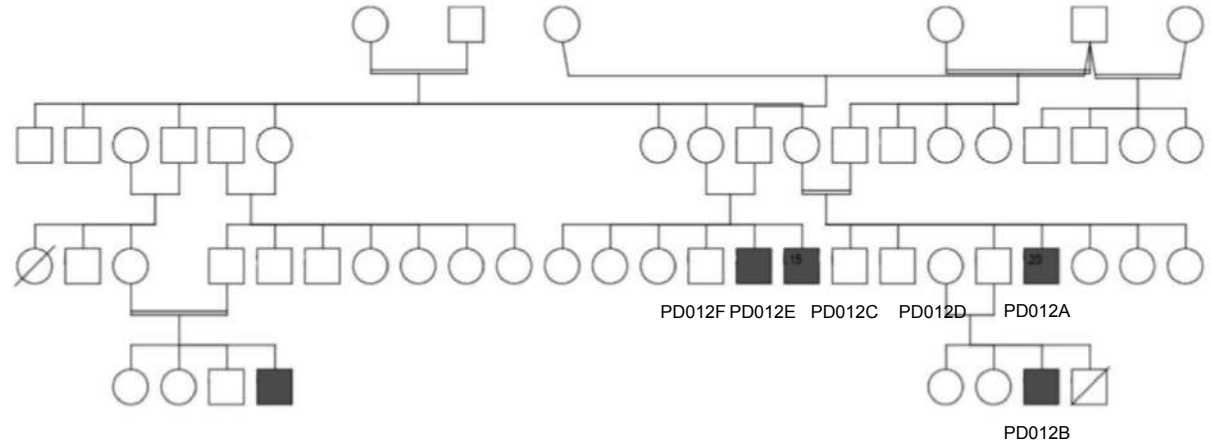
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PD012D
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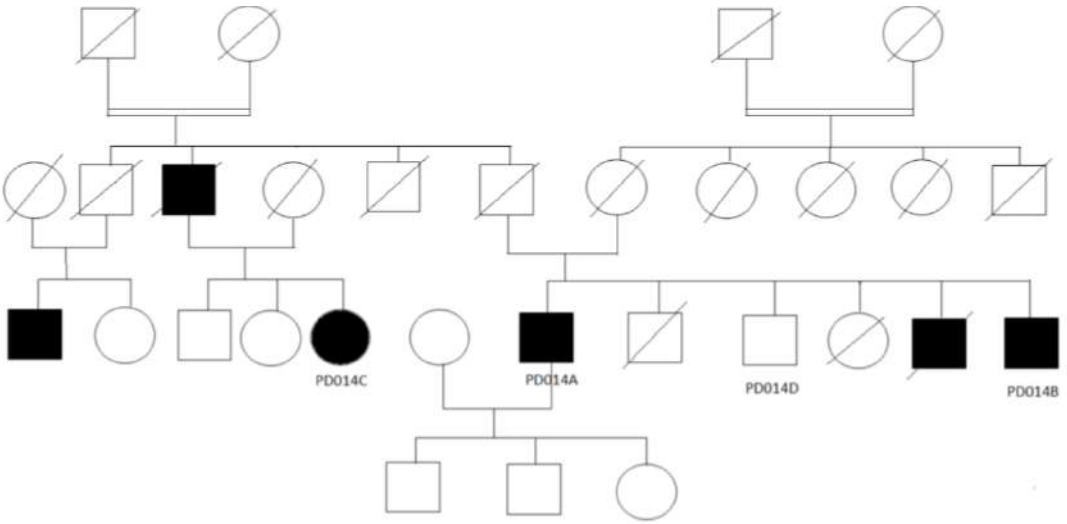
PD012B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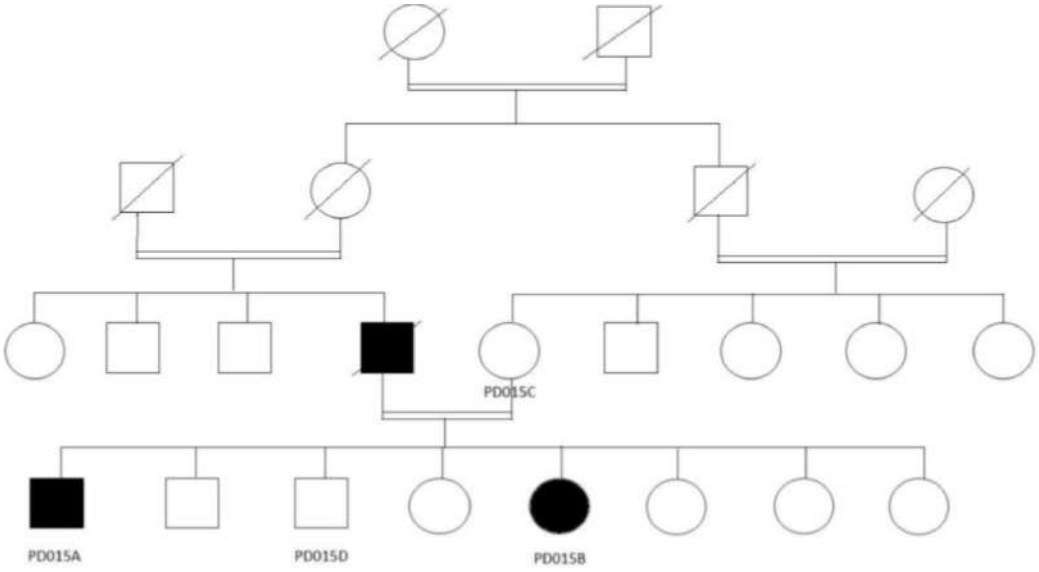
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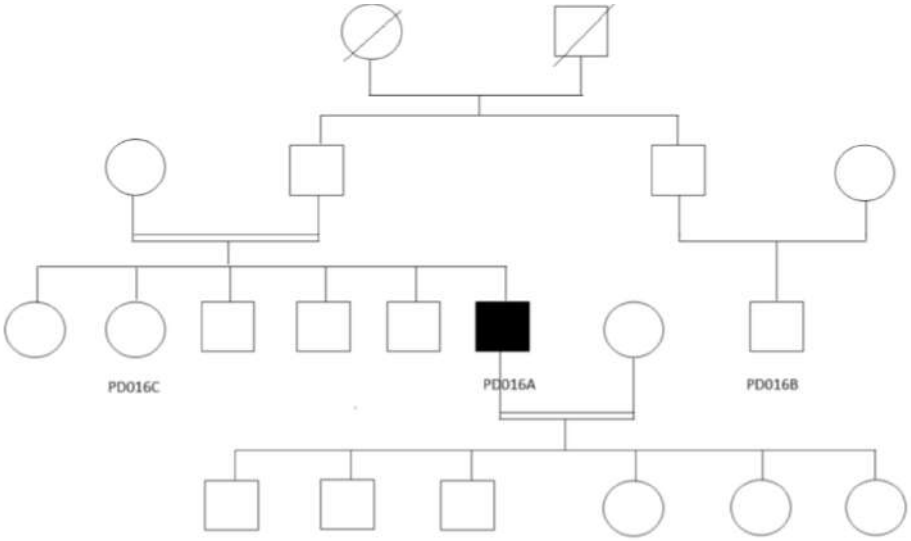
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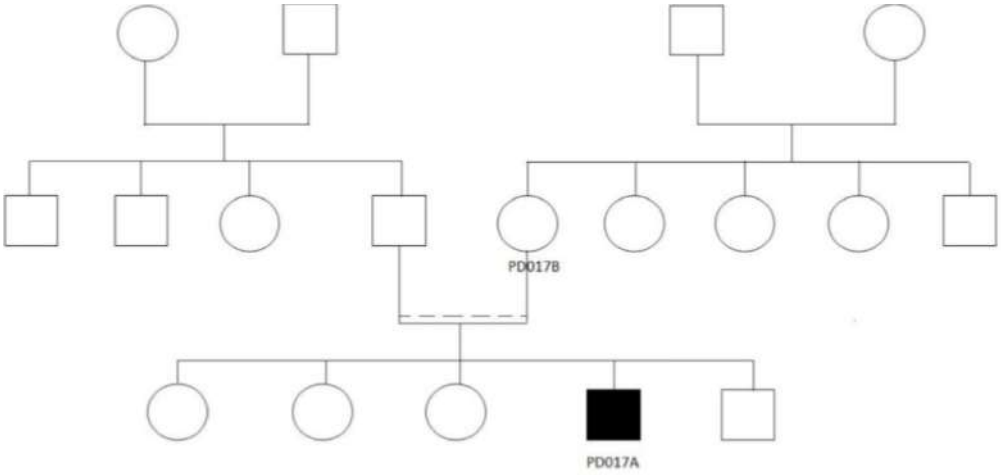
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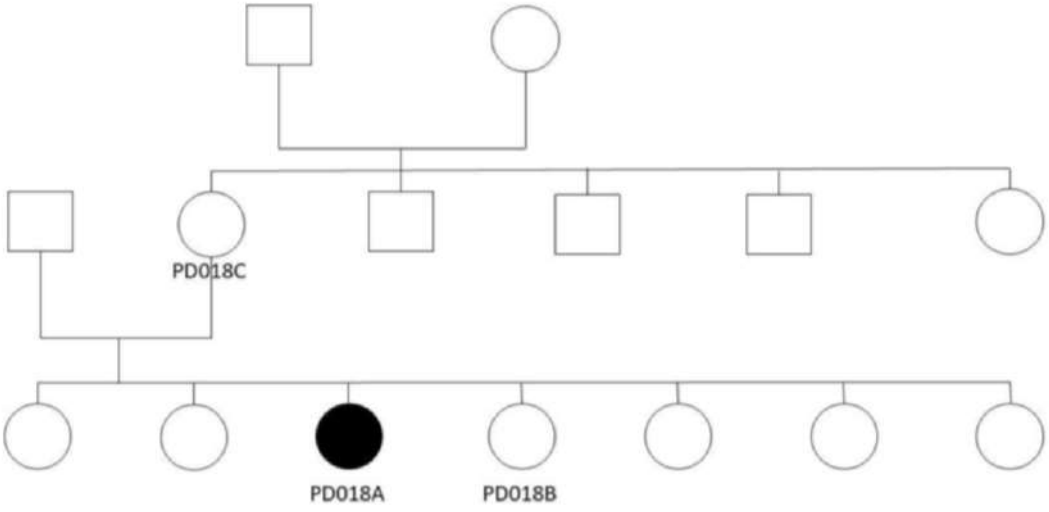
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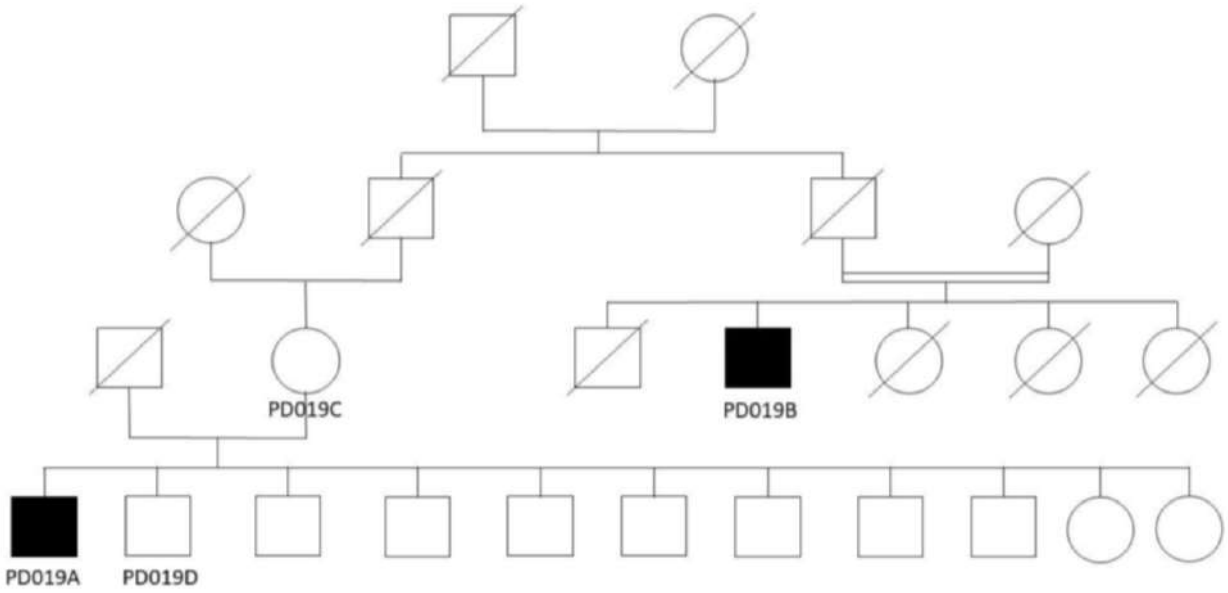
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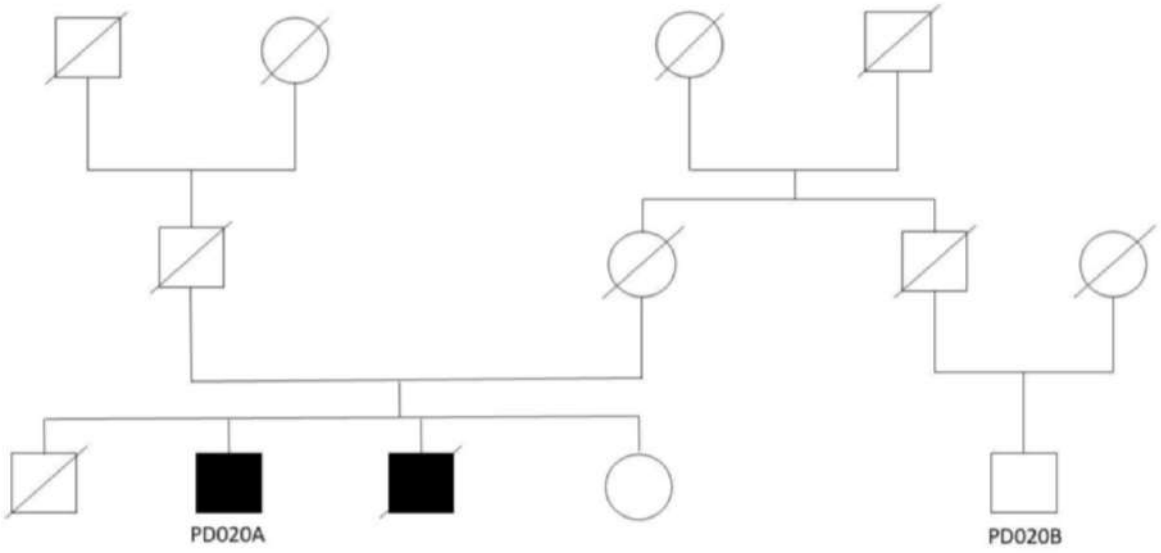
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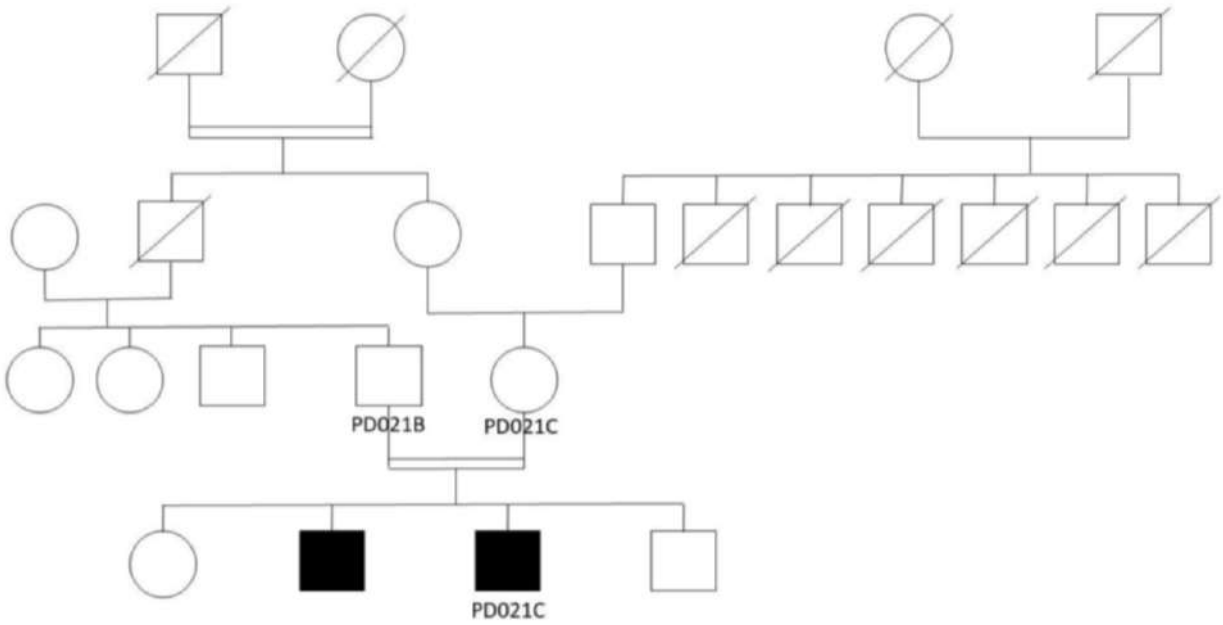
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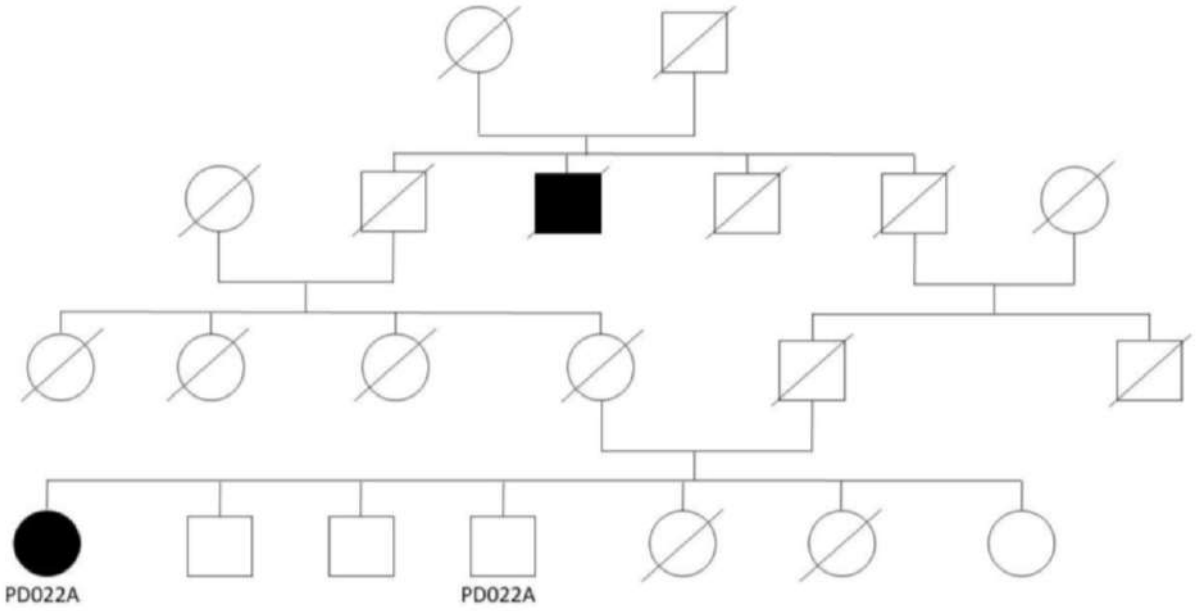
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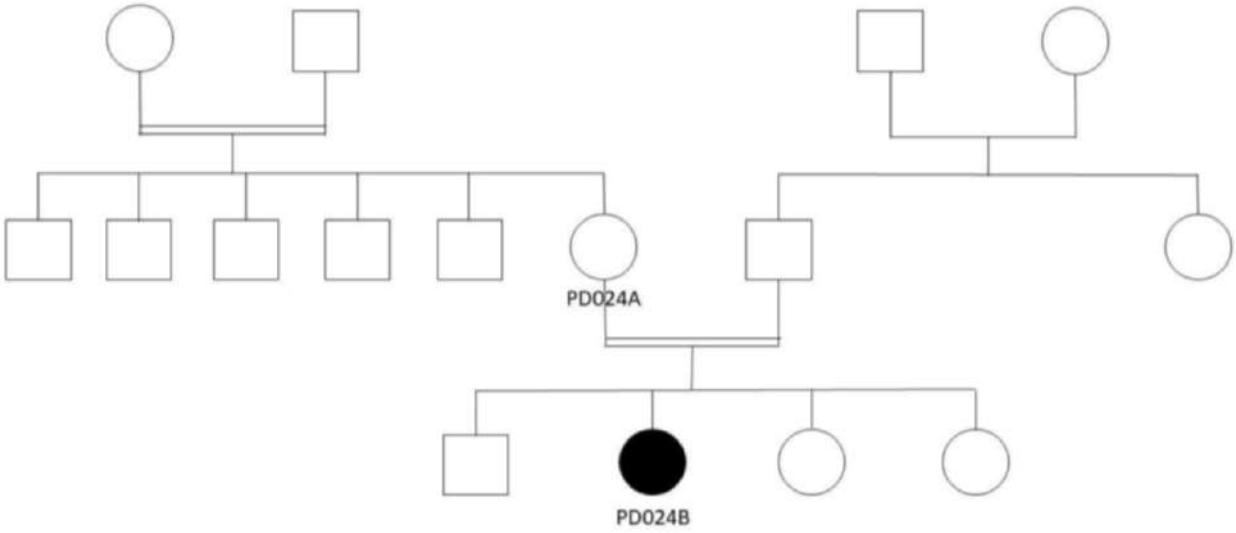
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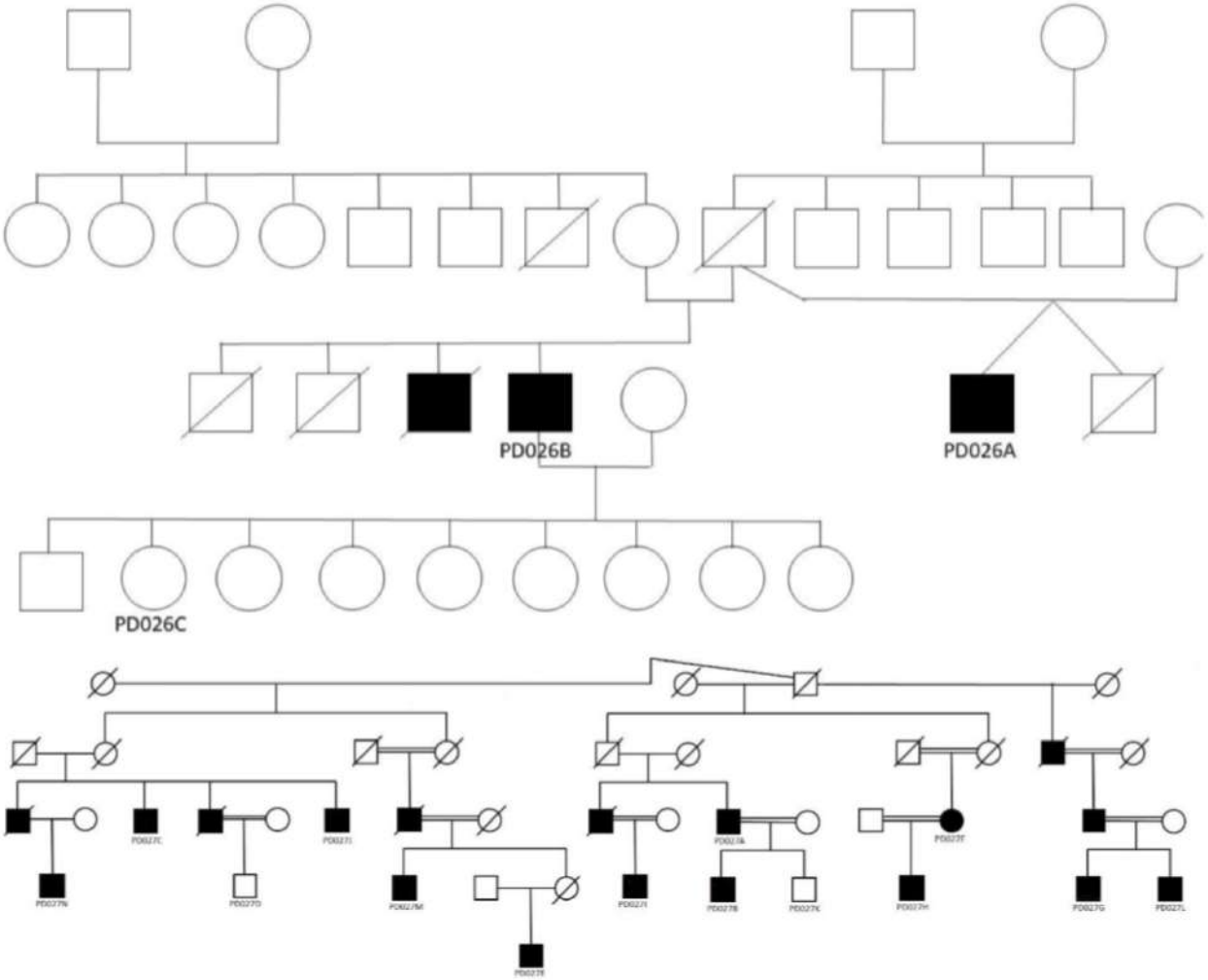
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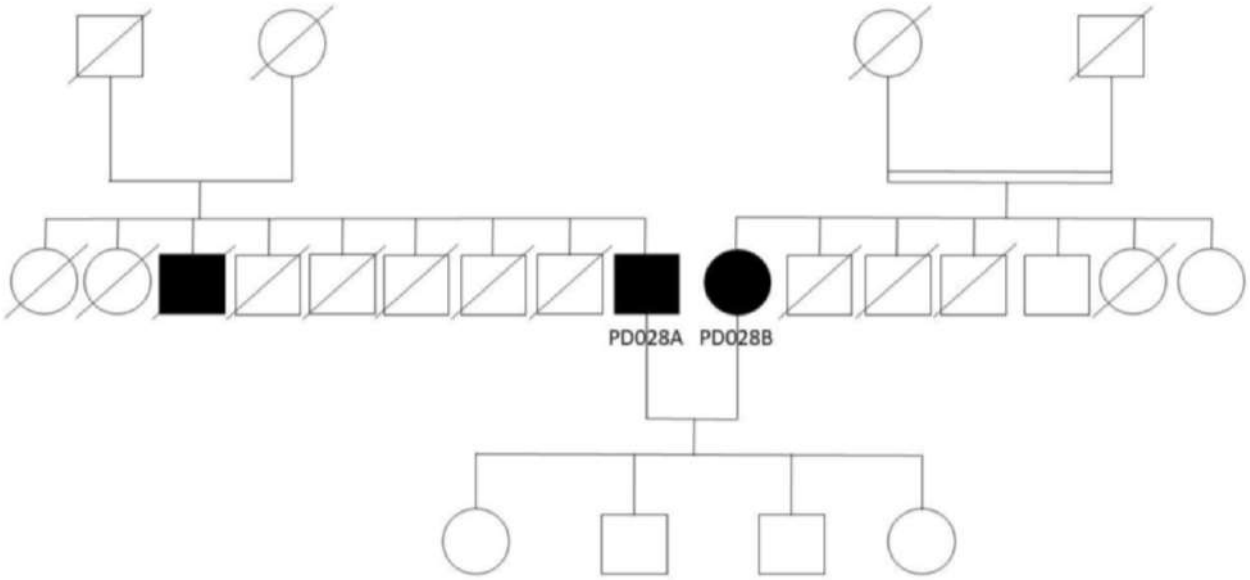
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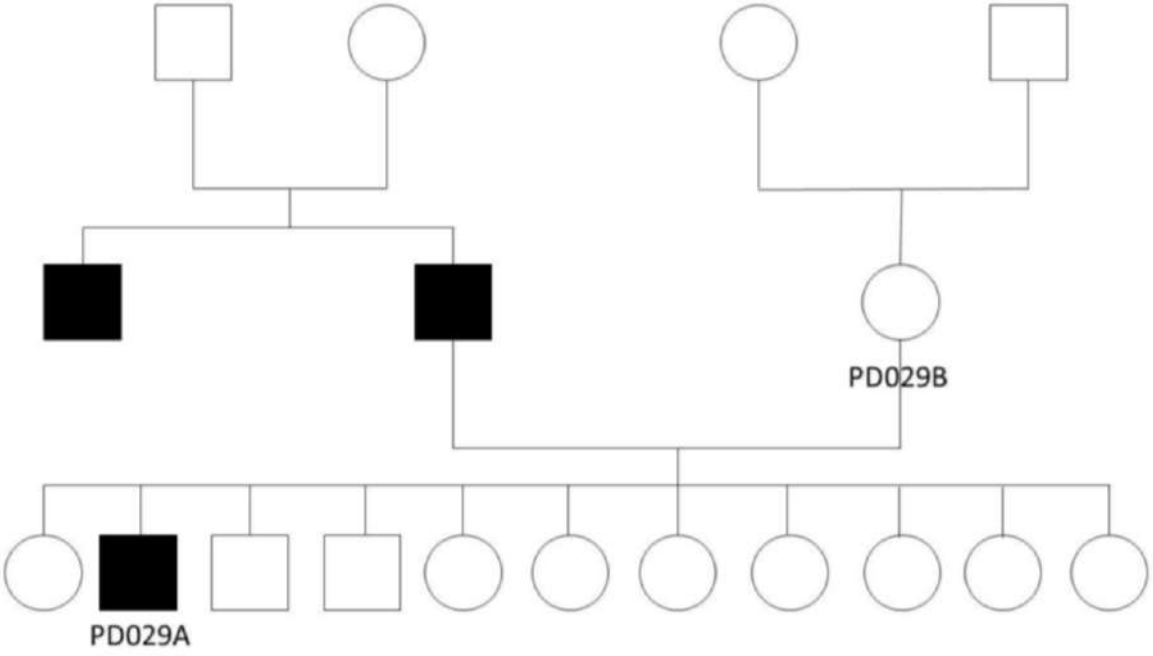
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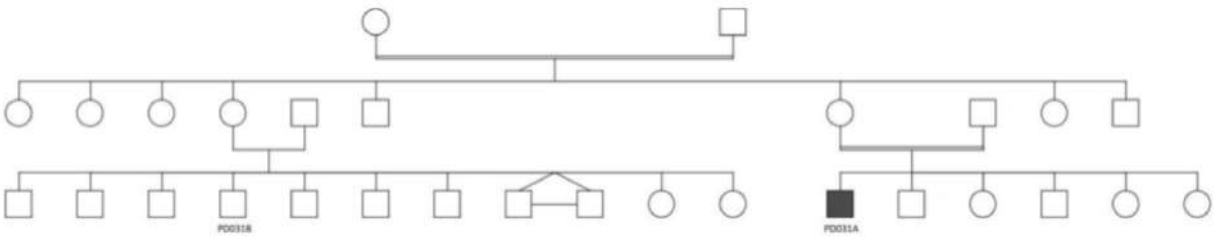

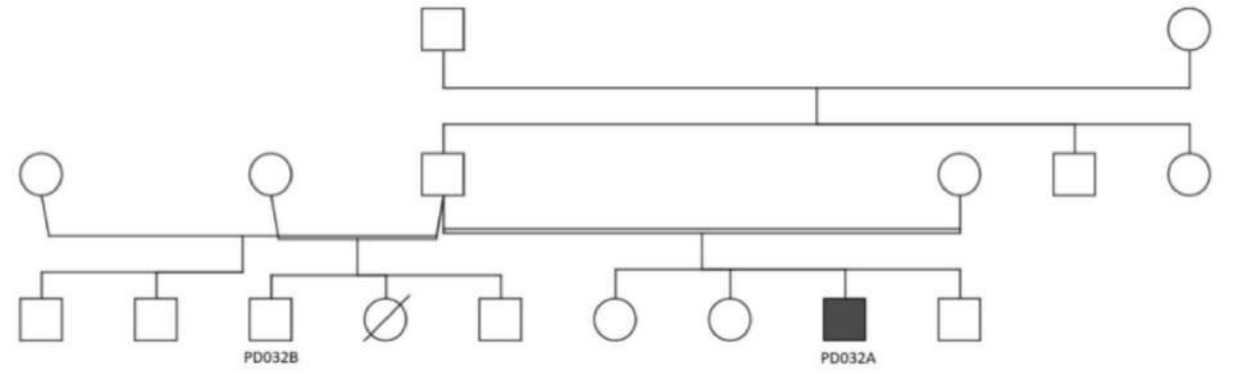
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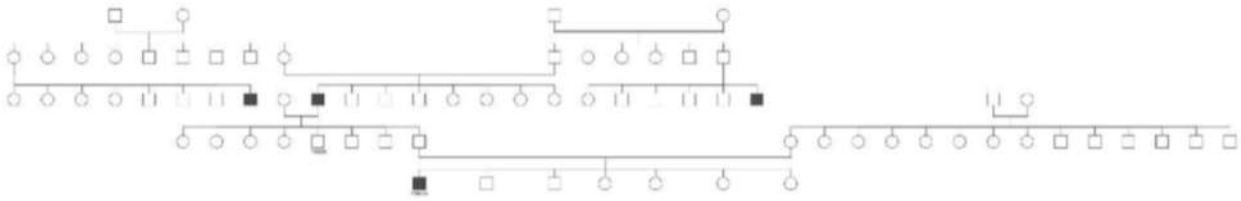

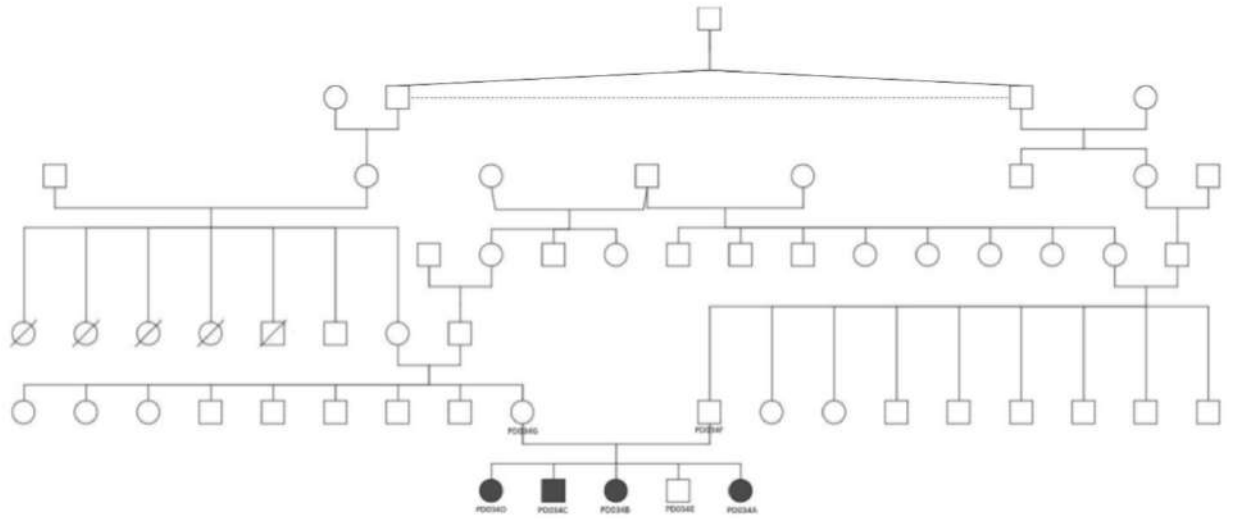

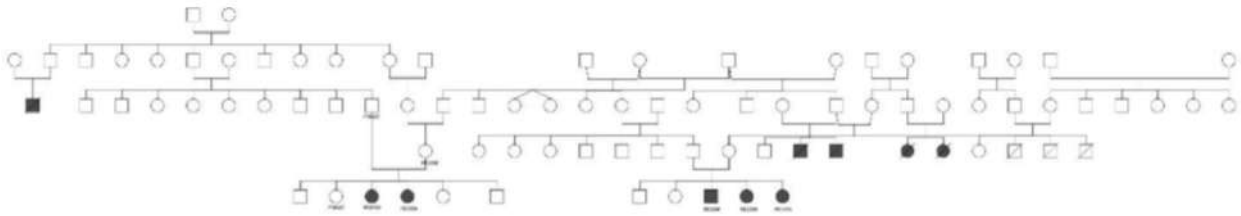

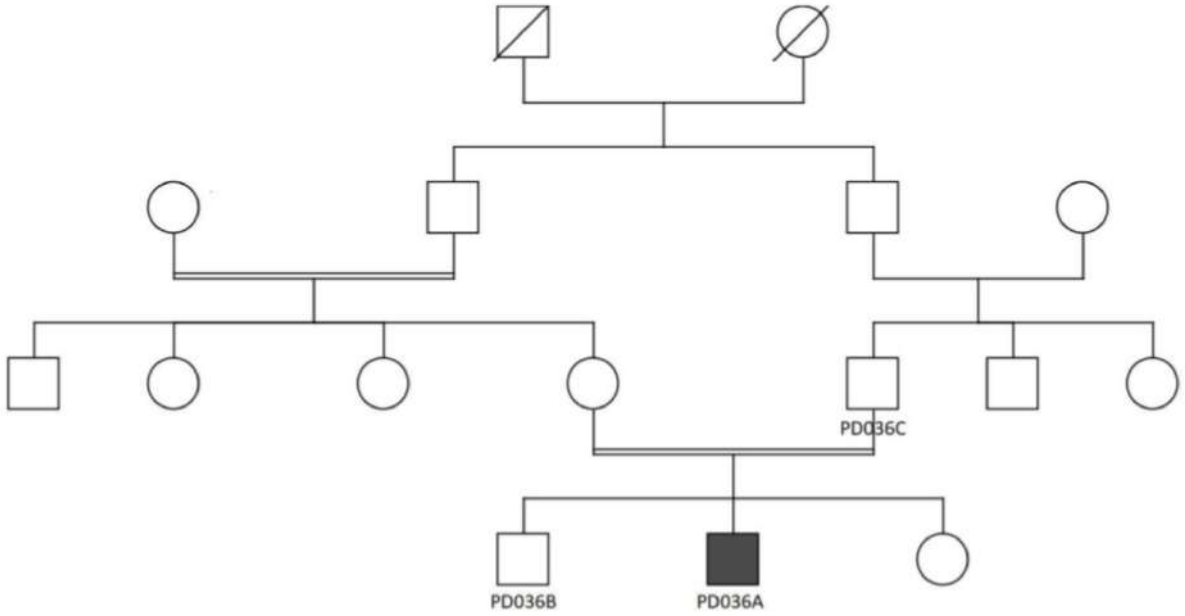
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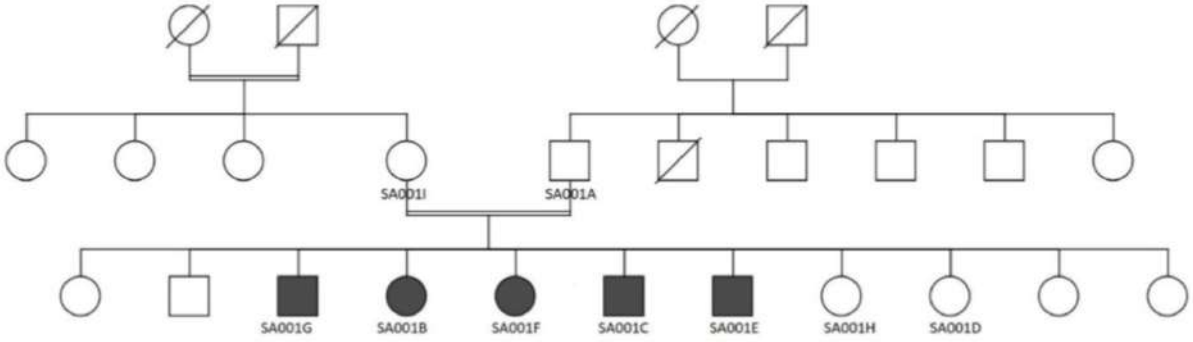
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SA001D

3.5.2: Summary of genetic findings

Table 1: summary of genetic findings with comfirmed segregation

Chromosome	gene	variant type	variant name	family	zygocity
6	PRKN	Exon deletion	PRKN: Ex 1 del	PD012	Htz
6	PRKN	Exon deletion	PRKN: Ex 6 del	PD012	Htz
1	DNAJC6	Non synonymous	rs78141380	PD025	Hom
14	SYNE2	nonframeshift substitution	SYNE2:NM_015180:exon61:c.12001_12002CA	PD009, PD011, PD019, PD020, PD021	Htz
X	PGK1	nonsynonymous SNV	PGK1:NM_000291:exon1:c.20T>C:p.L7P	PD032	Hom
17	POLR2A	frameshift deletion	POLR2A:NM_000937:exon30:c.5511delC:p.P1837fs	PD014	Hom
20	SLC52A3	nonsynonymous SNV	SLC52A3:NM_033409:exon5:c.1328G>C:p.G443A	PD033	Htz
20	SLC52A3	nonsynonymous SNV	SLC52A3:NM_033409:exon3:c.710C>T:p.A237V	PD033	Htz
2	NBAS	nonsynonymous SNV	NBAS:NM_015909:exon34:c.4035C>G:p.C1345W	PD035	Hom

Table 2: summery of genetic finding with probable effect and unconcluded analysis

Chromosome	gene	variant type	variant name	family	zygocity
21	SON	frame shift insertion	SON:NM_001291412:exon11:c.1321dupG:p.A441fs	PD009, PD010, PD011, PD014, PD016, PD017, PD019; PD022, PD023, PD024; PD026, PD027; PD028, PD029,PD030, PD031, PD032, PD033	hom
21	SON	frame shift deletion	SON:NM_001291412:exon11:c.1333delC:p.P445fs	PD009, PD010, PD011, PD014, PD016, PD017, PD019; PD022, PD023, PD024, PD026; PD027, PD028, PD029, PD030, PD031,PD032, PD033	hom

21	SON	frame shift insertion	SON:NM_001291412:exon11:c.1321dupG:p.A441fs	PD026	comp
21	SON	frame shift deletion	SON:NM_001291412:exon11:c.1333delC:p.P445fs	PD026	comp
14	SYNE2	frame shift insertion	SYNE2:NM_015180:exon22:c.2649dupA:p.E883fs	PD009, PD010, PD011, PD015, PD016, PD018, PD019, PD020,PD021, PD022; PD023, PD024, PD025, PD030, PD031, PD033	comp
14	SYNE2	frame shift deletion	SYNE2:NM_015180:exon12:c.1133delA:p.N378fs	PD010, PD011, PD015, PD017; PD018, PD019, PD020; PD022, PD023, PD024, PD025, PD029, PD031, PD032, PD032	comp
14	SYNE2	frame shift deletion	SYNE2:NM_015180:exon12:c.1132_1133del:p.N378fs	PD011, PD016, PD030	comp
14	SYNE2	frame shift insertion	SYNE2:NM_015180:exon22:c.2649dupA:p.E883fs	PD024	hom
14	SYNE2	frame shift deletion	SYNE2:NM_015180:exon12:c.1133delA:p.N378fs	PD024	hom
10	PTEN	frame shift deletion	PTEN:NM_001304717:exon2:c.155delG:p.R52fs	PD009, PD010, PD011, PD014, PD016, PD017, PD019, PD020, PD022, PD023, PD024, PD026, PD027; PD028, PD029, PD030, PD031, PD032, PD033	hom
1	MTR	frame shift deletion	MTR:NM_001291940:exon19:c.826delA:p.I276fs	PD010	COMP

1	MTR	frame shift insertion	MTR:NM_001291940:exon19:c.826dupA:p.I276fs	PD019	COMP
1	MTR	frame shift deletion	MTR:NM_001291939:exon23:c.2329delG:p.G777fs	PD010, PD019	COMP
13	COG6	frame shift deletion	COG6:NM_015145079:exon17:c.1693delG:p.G565fs	PD021	HOM
13	COG6	frame shift insertion	COG6:NM_015145079:exon7:c.628dupG:p.E210fs	PD029	HOM
13	COG6	frame shift insertion	COG6:NM_015145079:exon7:c.624dupT:p.G208fs	PD033	HOM
2	CPS1	nonframeshift substitution	CPS1:NM_001875:exon10:c.1030_1032GCT	PD023; PD025	HTZ

Table 3: summary of genes associated neurological phenotypes

Gene	Phenotype
PRKN	Parkinson's disease 2
DNAJC6	Parkinson's disease 19
SYNE2	Emery-Dreifuss musclular dystrophy intellectual disability and autism
PGK1	Stroke, intellectual disablility episodic muscle weakness of unknown etiology
POLR2A	Infantile onset hypotonia Glioblastoma, Neurodevelobmental disorder with intellectual and behavioural abnormality
SLC52A3	Brown-Vialetto-Van Laere syndrome, Fazio-Lunde disease
NBAS	Optic nerve atrophy
SON	ZTTK syndrome
PTEN	Macrocephaly/ Autism syndrome, meningioma
MTR	Neural tube defect
CPS1	Urea cycle deficiency induced tremor and ataxia

4. Discussion

4.1 Diversity consanguinity and the use of NGS in Parkinsonism from Sudan

Four genes were associated with familial cases which were identified with the disease panel; PINK1, PRKN1, PLA2G6 , and DNAJC6. The lack of definitive genetic diagnosis for the majority of families could partially be due to the unclear pattern of inheritance, possible poligenicity or non-exonic variants only detectable with whole genome sequencing (WGS). This reflects the heterogeneity of Parkinsonism in Sudanese population, which mirrors the diverse Parkinson population in Africa generally and sub Saharan Africa in Particular (Dekker MCJ et al, 2020; Blanckenberg J, 2013)

Sudan is a country located in northeastern Africa that has a diverse population with various ethnic, linguistic, and cultural backgrounds. The heterogeneity of the Sudanese population can be attributed to several factors, including migration, trade, colonization, and intermarriage. Sudan has been a crossroads for migration and trade for centuries, with various populations moving in and out of the country (S H 2003). This is demonstrated with over 570 ethnic groups and 100 languages spoken. The largest ethnic groups in Sudan include the Arab, Beja, Fur, Nubian, and Zaghawa, however, majority of Sudanese Arabs are of mixed Arab and indigenous African ancestry and live in the northern part of the country The Beja, Fur, Nubian, and Zaghawa are indigenous African ethnic groups who live in different regions of Sudan and speak distinct languages (A A-S, 1997; M J, 2007).

On the other hand, it is widely accepted that consanguineous marriages have been shown to increase the risk of genetic disorders, including inherited metabolic disorders and recessive genetic disorders. In Sudan, the prevalence of consanguineous marriages is among the highest, with rates ranging from 40% to 60% in some regions of the country

(Elamin W, 2017). This high rate of consanguinity is believed to contribute to the high prevalence of genetic disorders in Sudan. For example, sickle cell disease, which is an autosomal recessive disorder, has a prevalence of 0.3-2.0% in Sudan and is more common in regions where consanguinity rates are high (Elhassan, I et al 2015). Similarly other recessive disorders such as thalassemia and glucose-6-phosphate

dehydrogenase deficiency are also more prevalent in Sudan due to consanguineous marriages (Yang Y et al, 2013). Similar ongoing researches have re-affirmed the same role of consanguinity in recessive neurological disorders, therefore, it is crucial to raise awareness about the potential risks of consanguineous marriages and encourage genetic counseling to help reduce the incidence of genetic disorders in Sudan. Thus, by plotting the high consanguinity rate in Sudan and the genetic diversity of its ethnic groups, one can assume that the genetic characteristic of certain ethnic group will follow in most cases the ethnic and linguistic compartmentalization. As a result, we could expect multiple founder effects when studying a particular condition, taking into consideration that the rate of recessive disorders, hereditary conditions with reduced penetrance and atypical mendelian disorders would be relatively high.

Rare diseases, by definition, affect fewer than 1 in 2000 individuals. There are an estimated 7000 rare diseases that affect approximately 400 million individuals globally Patients with rare neurological disorders face several challenges, such as diagnosis, treatment, and access to healthcare services, these challenges are more patents in Sub Saharan Africa. However, recent advances in genomics, especially the development of next-generation sequencing (NGS) technologies, have provided a powerful tool to diagnose rare diseases. NGS technologies enable the analysis of the entire genome or specific regions of interest, making it possible to identify mutations and other genetic variants that cause these disorders. Whole-exome sequencing (WES) is a targeted sequencing approach that captures the coding regions of the genome, which account for approximately 1-2% of the entire genome. Whole-genome sequencing (WGS), on the other hand, sequences the entire genome, including the coding and non-coding regions. Both WES and WGS have been used successfully to diagnose rare neurological disorders.

In a study conducted by Yang et al., WES was used to diagnose patients with intellectual disability (ID) and developmental delay (DD). The study found that WES had a diagnostic yield of 29.5% in patients with ID and DD, and identified mutations in known disease-causing genes and novel candidate genes. Similarly WGS has been used to diagnose rare diseases, such as rare pediatric diseases, mitochondrial diseases, and cancer. In a study conducted by Li et al., WGS was used to diagnose a patient with a

rare mitochondrial disease, identifying a pathogenic variant in the MT-ND1 gene (Alvarez-Mora M I, 2023).

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In addition to WES and WGS, other NGS technologies, such as targeted gene panel sequencing (TGPS) and RNA sequencing, have also been used to diagnose rare diseases. Targeted gene panel sequencing involves sequencing a panel of genes associated with a particular disease or phenotype. RNA sequencing, on the other hand, enables the analysis of gene expression patterns and can be used to identify aberrant gene expression that causes rare diseases.

It is worth mentioning that NGS technologies pose several challenges and limitations to their use. One of the major challenges is the interpretation of the vast amounts of data generated by NGS. The identification of pathogenic variants from NGS data requires expertise in bioinformatics and functional genomics. Another challenge is the cost of NGS, which can be prohibitively expensive for some patients and healthcare systems. Additionally NGS can generate incidental findings that are not related to the patient's clinical phenotype, leading to ethical and legal issues. This is why it would be adviced that WES and WGS could be utilized in research oriented approach when identifying the genetic basis of a particular disease, which would then guide the development of custom made TGPS, which is affordable for a third world country like Sudan to be used as a diagnostic tool for rare genetic disorders in general and genetic neurological disorders in particular.

In this study the targeted NGS panel identified several known and novel mutations in six families out of thirty-six, which constitutes a 20% diagnostic rate of the disease panel, which is within the rate of diagnostic yield described before (Hyo Eun Moon SHP, 2015). Additionally the average number of indexed patients per family which were diagnosed with the panel is 3.7+/- 2.3, while for the unsolved families is 1.8+/-1.5, indicating that the targeted gene panel would be an appropriate tool to investigate families with two or more affected indexed members. TGPS and WES are two complementary approaches for identifying genetic variants in patients with suspected genetic disorders. TGPS is more cost-effective and faster, while WES provides a comprehensive view of the genetic variation in a patient and can identify variants in novel genes. The choice between these

approaches depends on the specific clinical question being addressed, the availability of disease-associated genes in existing targeted panels, and the available resources for data analysis.

4.2 Mitochondrial VS Synuclein associated PD:

Mitochondrial dysfunction and abnormal accumulation of α-synuclein protein are two key pathological features of PD. Thus, one may hypothesize on different subtypes of PD based on underlying genetic causes: mitochondrial PD and synuclein associated PD and perhaps also a lysosomal subtype although this can be associated or summarized with the synuclein-associated PD.

Mitochondrial PD on the one hand constitutes rare genetic variant subtypes of PD caused by mutations in mitochondrial genes, including PINK1, PARKIN, and DJ-1 and mitochondrial abnormalities have been reported in Parkin-deficient mouse and fruit fly models of PD (Monzio Compagnoni G, 2020) These genes are involved in mitochondrial quality control and their dysfunction leads to impaired mitophagy resulting in the accumulation of dysfunctional mitochondria and oxidative stress. Patients with mitochondrial PD typically have an earlier age of onset, are more likely to exhibit non-motor symptoms, and have a slower disease progression compared to synuclein PD (SCHAPIRA AH V et al, 2020). Interestingly sporadic PD has also been associated with mitochondrial pathology as early as 20 years ago (Monzio Compagnoni G, 2020) and very recent work indicates that mitochondrial dysfunction is detectable prior to neuronal loss and alpha-synuclein fibril deposition, suggesting that mitochondrial dysfunction is one of the key drivers of early disease (Tomey CE et all, 2022).

Synuclein PD, first and foremost is caused by mutations in the SNCA gene, which encodes α-synuclein, identified first in the quest for causes of familial PD (Polymeropoulos MH et all, 1997). Interestingly as multiplications of the SNCA gene also cause familial with high penetrance, the amount of synuclein protein seems to play a maior role. This is the rationale for analysis of the epigenetic regulation of SNCA gene expression (Wüllner U, 2015). Similar to familial PD due to mutations in the SNCA gene, idiopathic PD, the most common form of PD, is characterized by the accumulation of α-synuclein protein in Lewy bodies, the pathological hallmark features of the disease.

Familial cases in the current study mostly presented with a recessive pattern, and as no case from Sudan carried SNCA mutations or duplication, PD in Sudanese families rather represents mitochondrial conditions. Indeed three of the four genes associated with familial cases are involved in mitochondrial functions: PINK1, PRKN1, PLA2G6; DNAJ C6 belongs to the evolutionarily conserved DNAJ/HSP40 family of proteins, which regulate molecular chaperone activity

Differentiating between mitochondrial and synuclein-associated PD might beimportant for disease-modifying therapies. Mitochondrial PD patients may benefit from mitochondrial-targeted therapies, such as coenzyme Q10 or idebenone, which can improve mitochondrial function and reduce oxidative stress. On the other hand, synuclein PD patients may benefit from therapies aimed at reducing α-synuclein accumulation or preventing its aggregation (Monzio Compagnoni G, 2020).

Finally it is empirical to consider the challengers of Genetic researches in Africa and Sudan in particular before designing any research project. Firstly the geopolitical situation of Sudan makes it very difficult to conduct any research from conceptual, logistic and financial points of view The geopolitical borders of Sudan are blurred, as they are very widely open and shared with seven countries, where it is very common for the distant Sudanese families to have second degrees and sometimes first-degree relatives, sometimes with no clear official documents and even dual nationalities. To overcome this issue we identified our cohort as Sudanese citizen living within the landscape of Sudan. The economic/political turbulence poses a challenge for sampling and patients assessments from a financial and safety aspects. In many cases we had to rely on our own personal funding to manage the project and during risky times (e.g. revolution and military coup) we had to minimize our transport through private vehicles and in most cases we followed (in patients houses visits) approach. Additionally the sanctions imposed by the US makes is very difficult to receive any form of external fund/ support which usually delay the research schedule substantially Secondly Due to the limited trained personnel and absence of electronic data of patients, we had to established the whole entry system which is concerned with patients demographic data, clinical, phenotypic and social data from the patients files, for this reason, we took the opportunity of this project to train young undergraduate and graduate enthusiast to help in collecting data and providing capacity building for them

Sudan remains an undiscovered region for biomedical researches, with a plethora of strong candidates in all aspect. Similar project would import a distinct mark for advancing research in General and in Neurology in particular, whether for the sake of the Sudanese community or the international science community

4.5 References

A, A.-S. (1997). Sudanese Arabs and the African Identity. *Journal of Asian and African Studies*, *32*(1), 81–98.

Alvarez-Mora, M. I., Rodríguez-Revenga, L., Jodar, M., Potrony, M., Sanchez, A., Badenas, C., … Madrigal, I. (2023). Implementation of Exome Sequencing in Clinical Practice for Neurological Disorders. *Genes*, *14*(4), 1–11. https://doi.org/10.3390/genes14040813

Bakhit, Y., Schmitt, I., Hamed, A., Ibrahim, E. A. A., Mohamed, I. N., El-Sadig, S. M., … Wüllner, U. (2022). Methylation of alpha-synuclein in a Sudanese cohort. *Parkinsonism and Related Disorders*, *101*(June), 6–8. https://doi.org/10.1016/j.parkreldis.2022.05.009

Balicza, P., Bencsik, R., Lengyel, A., Gal, A., Grosz, Z., Csaban, D., … Molnar, M. J. (2020). Novel dominant MPAN family with a complex genetic architecture as a basis for phenotypic variability. *Neurology: Genetics*, *6*(5). https://doi.org/10.1212/NXG.0000000000000515

Benamer, H. TS., de Silva, R., Siddiqui, K. A., & Grosset, D. G. (2008). Parkinson's disease in Arabs: A systematic review. *Movement Disorders*, *23*(9), 1205–1210. https://doi.org/10.1002/mds.22041

Blanckenberg, J., Bardien, S., Glanzmann, B., Okubadejo, N. U., & Carr, J. A. (2013). The prevalence and genetics of Parkinson's disease in sub-Saharan Africans. *Journal of the Neurological Sciences*, *335*(1–2), 22–25. https://doi.org/10.1016/j.jns.2013.09.010

Blauwendraat, C., Nalls, M. A., & Singleton, A. B. (2020). The genetic architecture of Parkinson's disease. *The Lancet Neurology*, *19*(2), 170–178.

https://doi.org/10.1016/S1474-4422(19)30287-X

Bloem, B. R., Okun, M. S., & Klein, C. (2021). Parkinson's disease. The Lancet, 397(10291), 2284–2303. https://doi.org/10.1016/S0140-6736(21)00218-X

Bonifati, V., Rohe´, G. ., Breedveld, E., Fabrizio, E., De Mari, M., & Tassorelli, C. (2006). Early-onset parkinsonism associated with PINK1 mutations: Frequency genotypes, and phenotypes [1]. Neurology, 66(7), 1129. https://doi.org/10.1212/01.wnl.0000220157.81513.85

Bonifati, Vincenzo. (2012). Autosomal recessive parkinsonism. Parkinsonism and Related Disorders, 18(SUPPL. 1), S4–S6. https://doi.org/10.1016/s1353-8020(11)70004-9

Cazeneuve, C., S??n, C., Ibrahim, S. A., Mukhtar, M. M., Kheir, M. M., LeGuern, E., … Salih, M. A. (2009). A new complex homozygous large rearrangement of the PINK1 gene in a Sudanese family with early onset Parkinson's disease. Neurogenetics, 10(3), 265–270. https://doi.org/10.1007/s10048-009-0174-4

Chen, X., Yuan, L., Sun, M., Liu, Q., & Wu, Y. (2018). Two novel CPS1 mutations in a case of carbamoyl phosphate synthetase 1 deficiency causing hyperammonemia and leukodystrophy. Journal of Clinical Laboratory Analysis, 32(5), 2–7. https://doi.org/10.1002/jcla.22375

Chishti, M. A., Bohlega, S., Ahmed, M., Loualich, A., Carroll, P., Sato, C., … Rogaeva, E. (2006). T313M PINK1 mutation in an extended highly consanguineous Saudi family with early-onset Parkinson disease. Archives of Neurology, 63(10), 1483–1485. https://doi.org/10.1001/archneur.63.10.1483

Dallapiccola, B. (1906). The PINK1 phenotype can be indistinguishable from idiopathic Parkinson disease. 20–22.

Daniel, S. E., & Lees, A. J. (1993). Parkinson's Disease Society Brain Bank, London: overview and research. Journal of Neural Transmission. Supplementum, 39, 165–172.

De Figueiredo, P., Drecktrah, D., Katzenellenbogen, J. A., Strang, M., & Brown, W. J.

(1998). Evidence that phospholipase A2 activity is required for Golgi complex and trans Golgi network membrane tubulation. *Proceedings of the National Academy of Sciences of the United States of America*, 95(15), 8642–8647. https://doi.org/10.1073/pnas.95.15.8642

De Figueiredo, P., Drecktrah, D., Polizotto, R. S., Cole, N. B., Lippincott-Schwartz, J., & Brown, W. J. (2000). Phospholipase A2 antagonists inhibit constitutive retrograde membrane traffic to the reticulum. *Traffic*, 1(6), 504–511. https://doi.org/10.1034/j.1600-0854.2000.010608.x

De Figueiredo, P., Polizotto, R. S., Drecktrah, D., & Brown, W. J. (1999). Membrane tubule-mediated reassembly and maintenance of the Golgi complex is disrupted by phospholipase A2 antagonists. *Molecular Biology of the Cell*, 10(6), 1763–1782. https://doi.org/10.1091/mbc.10.6.1763

Dekker, M. C. J., Coulibaly, T., Bardien, S., Ross, O. A., Carr, J., & Komolafe, M. (2020). Parkinson's Disease Research on the African Continent: Obstacles and Opportunities. *Frontiers in Neurology*, 11(June). https://doi.org/10.3389/fneur.2020.00512

Desplats, P., Spencer, B., Crews, L., Pathel, P., Morvinski-friedmann, D., Kosberg, K., … Masliah, E. (2012). □ -Synuclein Induces Alterations in Adult Neurogenesis in Parkinson Disease Models via p53-mediated Repression of. *Journal of Biological Chemistry*, 287(38), 31691–31702. https://doi.org/10.1074/jbc.M112.354522

DFT et al. (2018). Cerebrospinal fluid markers of central urea cycle metabolism correlate with disease severity in Huntington's disease. *Journal of Huntington's Disease*, 7(3), 259–269.

Drecourt, A., Babdor, J., Dussiot, M., Petit, F., Goudin, N., Garfa-Traoré, M., … Rötig, A. (2018). Impaired Transferrin Receptor Palmitoylation and Recycling in Neurodegeneration with Brain Iron Accumulation. *American Journal of Human Genetics*, 102(2), 266–277. https://doi.org/10.1016/j.ajhg.2018.01.003

Elamin, W. S., I. (2017). Consanguinity and genetic disorders in Sudan. *Sudanese*

Journal of Paediatrics, 17(2), 19–27.

Elhassan, I. M., Abbasher, D., Idris, S. A., Ali, M. E., Adam, I., & Eltahir, E. M. (2015). Prevalence and molecular characterization of glucose-6-phosphate dehydrogenase deficiency in Sudan. Hematology, 20(2), 112–117.

Elsayed, L. E. O., Mohammed, I. N., Hamed, A. A. A., Elseed, M. A., Salih, M. A. M., Yahia, A., … Stevanin, G. (2018). Case report of a novel homozygous splice site mutation in PLA2G6 gene causing infantile neuroaxonal dystrophy in a Sudanese family. BMC Medical Genetics, 19(1), 1–5. https://doi.org/10.1186/s12881-018-0592-y

Fahn, S. (2003). Description of Parkinson' s Disease as a Clinical Syndrome. Annals New York Academy of Sciences, 991, 1–14. https://doi.org/10.1111/j.1749-6632.2003.tb07458.x

Garrison, E., & Marth, G. (2012). Haplotype-based variant detection from short-read sequencing. 1–9. Retrieved from http://arxiv.org/abs/1207.3907

Gelb, D J, Oliver, E., & Gilman, S. (1999). Diagnostic criteria for Parkinson disease. Archives of Neurology, 56(1), 33–39. https://doi.org/10.1001/archneur.56.1.33

Gelb, Douglas J., Oliver, E., & Gilman, S. (1999). Diagnostic criteria for Parkinson disease. Archives of Neurology, 56(1), 33–39. https://doi.org/10.1001/archneur.56.1.33

Ghazavi, F., Fazlali, Z., Banihosseini, S. S., Hosseini, S. R., Kazemi, M. H., Shojaee, S., … Elahi, E. (2011). PRKN, DJ-1, and PINK1 screening identifies novel splice site mutation in PRKN and two novel DJ-1 mutations. Movement Disorders, 26(1), 80–89. https://doi.org/10.1002/mds.23417

Giri, A., Guven, G., Hanagasi, H., Hauser, A. K., Erginul-Unaltuna, N., Bilgic, B., … Simón-Sánchez, J. (2016). PLA2G6 mutations related to distinct phenotypes: A new case with early-onset parkinsonism. Tremor and Other Hyperkinetic Movements, 2016, 1–6. https://doi.org/10.7916/D81G0M12

Goetz, C. G., Tilley, B. C., Shaftman, S. R., Stebbins, G. T., Fahn, S., Martinez-Martin,

P., … Zweig, R. M. (2008). Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Scale presentation and clinimetric testing results. *Movement Disorders*, *23*(15), 2129–2170. https://doi.org/10.1002/mds.22340

Gregg, E. W., & Shaw, J. E. (2017). Global Health Effects of Overweight and Obesity *New England Journal of Medicine*, *377*(1), 80–81. https://doi.org/10.1056/nejme1706095

Guillermo Garcia-Manero Shao-Qing Kuang, Susan O'Brien, Deborah Thomas, and Hagop Kantarjian, H. Y., Michael R. Bronsert, William G. Henderson, Robert Valuck, Patrick Hosokawa, and K. H., Jones, R., Sheean, Hongmei Cao, Rui Yu, Yi Tao, Dejan Nikolic, and R. B. van B., Sheean, & Jones, R. (2014). 基因的改变 *NIH Public Access*. *Bone*, *23*(1), 1–7. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf

Hayflick, S. J., Kurian, M. A., & Hogarth, P. (2018). Neurodegeneration with brain iron accumulation. *Handbook of Clinical Neurology*, *147*, 293–305. https://doi.org/10.1016/B978-0-444-63233-3.00019-1

Herman, H., & Shambaugh, E. (2018). *Shambaugh1977*. (February), 2083–2087.

Hernandez, D. G., Reed, X., & Singleton, A. B. (2016). Genetics in Parkinson disease: Mendelian versus non-Mendelian inheritance. *Journal of Neurochemistry*, *139*, 59–74. https://doi.org/10.1111/jnc.13593

Hyo Eun Moon, S. H. P. (2015). Mitochondrial Dysfunction in Parkinson's Disease. *Experimental Neurobiolgy*, *24*(2), 103–116.

Ibrahim, M. E. (2021). Genetic diversity of the Sudanese: Insights on origin and implications for health. *Human Molecular Genetics*, *30*(R1), R37–R41. https://doi.org/10.1093/hmg/ddab028

Jost, W. H., & Reichmann, H. (2017). "An essay on the shaking palsy" 200 years old. *Journal of Neural Transmission*, *124*(8), 899–900. https://doi.org/10.1007/s00702-017-1684-0

Jowaed, A., Schmitt, I., Kaut, O., & Wüllner, U. (2010). Methylation regulates alpha-synuclein expression and is decreased in Parkinson's disease patients' brains. *Journal of Neuroscience*, 30(18), 6355–6359. https://doi.org/10.1523/JNEUROSCI.6119-09.2010

Kaltenboeck, A., Johnson, S. J., Davis, M. R., Birnbaum, H. G., Carroll, C. A., Tarrants, M. L., & Siderowf, A. D. (2012). Direct costs and survival of medicare beneficiaries with early and advanced parkinson's disease. *Parkinsonism and Related Disorders*, 18(4), 321–326. https://doi.org/10.1016/j.parkreldis.2011.11.015

Karkheiran, S., Shahidi, G. A., Walker, R. H., Paisa, C., Clinic, M. D., Hospital, H. R., … Institutes, D. (n.d.). Case Reports PLA2G6 -associated Dystonia – *Parkinsonism : Case Report and Literature Review*. https://doi.org/10.7916/D84Q7T4W

Keyser, R. J., Lesage, S., Brice, A., Carr, J., & Bardien, S. (2010). Assessing the prevalence of PINK1 genetic variants in South African patients diagnosed with early- and late-onset Parkinson's disease. *Biochemical and Biophysical Research Communications*, 398(1), 125–129. https://doi.org/10.1016/j.bbrc.2010.06.049

Khateeb, S., Flusser, H., Shelef, I., Narkis, G., Vardi, G., Shorer, Z., … Birk, O. S. (2006). Shareef Khateeb, * Hagit Flusser, * Rivka O r, Ilan Shelef, Ginat Narkis, Gideon Vardi, Zamir Shorer, Rachel Levy Aharon Galil, Khalil Elbedour, and Ohad S. Birk. *Journal of Human Genetics*, 79(November), 942–948.

Kitada, T., Aakawa, S., Hattori, N., Matsumine, H., Yokochi, M., Mizuno, Y., & Shimizu, N. (1998). Mutations in the. *Nature Letters*, 169(1993), 166–169. Retrieved from https://www.nature.com/articles/33416.pdf

Klein, C., Djarmati, A., Hedrich, K., Schäfer, N., Scaglione, G., Marchese, R., … Pramstaller, P. P. (2005). PINK1, Parkin, and DJ-1 mutations in Italian patients with early-onset parkinsonism. *European Journal of Human Genetics*, 13(9), 1086–1093. https://doi.org/10.1038/sj.ejhg.5201455

L. Ishihara-Paul, M.M. Hulihan, J. Kachergus, R. Upmanyu, L. Warren, & R. Amouri.

(2008). mutations and parkinsonism. Neurolo.

Larsson Forsell, PK. A., Kennedy B. P, & Claesson, H. E. (1999). The human calcium-independent phospholipase A2 gene: Multiple enzymes with distinct properties from a single gene. European J ournal of Biochemistry, 262(2), 575–585. https://doi.org/10.1046/j.1432-1327.1999.00418.x

Leutenegger, A. L., Salih, M. A. M., Ibáñez, P, Mukhtar, M. M., Lesage, S., Arabi, A., … Brice, A. (2006). Juvenile-onset parkinsonism as a result of the first mutation in the adenosine triphosphate orientation domain of PINK1. Archives of Neurology, 63(9), 1257–1261. https://doi.org/10.1001/archneur.63.9.1257

Li, H., & Durbin, R. (2010). Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics, 26(5), 589–595. https://doi.org/10.1093/bioinformatics/btp698

Li, H., Handsaker, B., Wysoker, A., Fennell, T, Ruan, J., Homer, N., … Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. Bioinformatics, 25(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Li, J. Y, Englund, E., Holton, J. L., Soulet, D., Hagell, P, Lees, A. J., … Brundin, P (2008). Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. Nature Medicine, 14(5), 501–503. https://doi.org/10.1038/nm1746

Lin, G., Lee, PT, Chen, K., Mao, D., Tan, K. L., Zuo, Z., … Bellen, H. J. (2018). Phospholipase PLA2G6, a Parkinsonism-Associated Gene, Affects Vps26 and Vps35, Retromer Function, and Ceramide Levels, Similar to α-Synuclein Gain. Cell Metabolism, 28(4), 605-618.e6. https://doi.org/10.1016/j.cmet.2018.05.019

Liu L, et al. (2020). Inhibition of CPS1 ameliorates neuroinflammation and oxidative stress in Parkinson's disease. Biochemical and Biophysical Research Communications, 521(1), 145–151.

M, J. (2007). Sudan: Race, Religion, and Violence. Polity Press.

Magrinelli, F, Mehta, S., Di Lazzaro, G., Latorre, A., Edwards, M. J., Balint, B., …

Bhatia, K. P(2022). Dissecting the Phenotype and Genotype of PLA2G6-Related Parkinsonism. Movement Disorders, 37(1), 148–161. https://doi.org/10.1002/mds.28807

Matsumoto, L., Takuma, H., Tamaoka, A., Kurisaki, H., Date, H., Tsuji, S., & Iwata, A. (2010). CpG demethylation enhances alpha-synuclein expression and affects the pathogenesis of Parkinson's disease. PLoS ONE, 5(11), 1–9. https://doi.org/10.1371/journal.pone.0015522

Michelis, J. P., Hattingen, E., Gaertner, FC., Minnerop, M., Träber, F., Biskup, S., … Paus, S. (2017). Expanded phenotype and hippocampal involvement in a novel compound heterozygosity of adult PLA2G6 associated neurodegeneration (PARK14). Parkinsonism and Related Disorders, 37, 111–113. https://doi.org/10.1016/j.parkreldis.2017.01.005

Monzio Compagnoni, G., Di Fonzo, A., Corti, S., Comi, G. P., Bresolin, N., & Masliah, E. (2020). The Role of Mitochondria in Neurodegenerative Diseases: the Lesson from Alzheimer's Disease and Parkinson's Disease. Molecular Neurobiology, 57(7), 2959–2980. https://doi.org/10.1007/s12035-020-01926-1

Myhre, R., Steinkjer, S., Stormyr, A., Nilsen, G. L., Abu Zayyad, H., Horany K., … Klungland, H. (2008). Significance of the parkin and PINK1 gene in Jordanian families with incidences of young-onset and juvenile parkinsonism. BMC Neurology, 8, 47. https://doi.org/10.1186/1471-2377-8-47

Oertel, W., & Schulz, J. B. (2016). Current and experimental treatments of Parkinson disease: A guide for neuroscientists. Journal of Neurochemistry, 139, 1–13. https://doi.org/10.1111/jnc.13750

Okubadejo, N. U., Bower, J. H., Rocca, W. A., & Maraganore, D. M. (2006). Parkinson's disease in Africa: A systematic review of epidemiologic and genetic studies. Movement Disorders, 21(12), 2150–2156. https://doi.org/10.1002/mds.21153

Osterhaus, A., Groen, J., Bildt, M. Van De, Martina, B., Vos, J., & Egmond, H. Van. (1997). a -Synuclein in Lewy bodies. Nature, 388, 839–840.

Paisán-Ruiz, C., Guevara, R., Federoff, M., Hanagasi, H., Sina, F., Elahi, E., … Houlden, H. (2010). Early-onset L-dopa-responsive Parkinsonism with pyramidal signs due to ATP13A2, PLA2G6, FBXO7 and Spatacsin mutations. Movement Disorders, 25(12), 1791–1800. https://doi.org/10.1002/mds.23221

Paisán-Ruiz, C., Li, A., Schneider, S. A., Holton, J. L., Johnson, R., Kidd, D., … Houlden, H. (2012). Widespread Lewy body and tau accumulation in childhood and adult onset dystonia-parkinsonism cases with PLA2G6 mutations. Neurobiology of Aging, 33(4), 814–823. https://doi.org/10.1016/j.neurobiolaging.2010.05.009

Pereira, L., Mutesa, L., Tindana, P., & Ramsay M. (2021). African genetic diversity and adaptation inform a precision medicine agenda. Nature Reviews Genetics, 22(May). https://doi.org/10.1038/s41576-020-00306-8

Pettersen, E. F., Goddard, T. D., Huang, C. C., Meng, E. C., Couch, G. S., Croll, T. I., … Ferrin, T. E. (2021). UCSF ChimeraX: Structure visualization for researchers, educators, and developers. Protein Science, 30(1), 70–82. https://doi.org/10.1002/pro.3943

Polymeropoulos, M. H., Lavedan, C., Leroy E., Ide, S. E., Dehejia, A., Dutra, A., … Nussbaum, R. L. (1997). Mutation in the α-synuclein gene identified in families with Parkinson's disease. Science, 276(5321), 2045–2047. https://doi.org/10.1126/science.276.5321.2045

Riku, Y., Ikeuchi, T., Yoshino, H., Mimuro, M., Mano, K., Goto, Y., … Yoshida, M. (2014). Extensive aggregation of α-synuclein and tau in juvenile-onset neuroaxonal dystrophy: An autopsied individual with a novel mutation in the PLA2G6 gene-splicing site. Acta Neuropathologica Communications, 2(1), 1–11. https://doi.org/10.1186/2051-5960-1-12

S, H. (2003). Ethnic Diversity and Conflict in Sudan: A Historical and Contemporary Overview. Mediterranean Quarterly, 14(3), 30–44.

Samii, A., Nutt, J. G., & Ransom, B. R. (2004). Parkinson's disease. Lancet (London, England), 363(9423), 1783–1793. https://doi.org/10.1016/S0140-6736(04)16305-

8

SCHAPIRA, A. H. V, COOPER, J. M., DEXTER, D., JENNER, P, CLARK, J. B., & MARSDEN, C. D. (1988). Mitochondrial complex I deficiency in Parkinson's disease. Lancet, I, 1289.

Schmidt, S. (2009). Measuring absorptive capacity Proceedings of the International Conference on Intellectual Capital, Knowledge Management & Organizational Learning, 254–260. https://doi.org/10.1101/gr.107524.110.20

Schmitt, I., Kaut, O., Khazneh, H., DeBoni, L., Ahmad, A., Berg, D., … Wüllner, U. (2015). L-dopa increases α-synuclein DNA methylation in Parkinson's disease patients in vivo and in vitro. Movement Disorders, 30(13), 1794–1801. https://doi.org/10.1002/mds.26319

Schneider, S. A., Hardy J., & Bhatia, K. P(2012). Syndromes of neurodegeneration with brain iron accumulation (NBIA): An update on clinical presentations, histological and genetic underpinnings, and treatment considerations. Movement Disorders, 27(1), 42–53. https://doi.org/10.1002/mds.23971

Seitelberger, F(1971). Neuropathological conditions related to neuroaxonal dystrophy Acta Neuropathologica, 5, 17–18. https://doi.org/10.1007/978-3-642-47449-1_3

Shan C, et al. (2021). Increased CPS1 expression is associated with Alzheimer's disease pathology Brain Research Bulletin, 169, 108–116.

Shantanam, S., & MUELLER. (2018). 乳鼠心肌提取 HHS Public Access. Physiology & Behavior, 176(1), 139–148. https://doi.org/10.1136/jmg.2008.063917.Parkin

Shi, C. H., Tang, B. S., Wang, L., Lv, Z. Y., Wang, J., Luo, L. Z., … Guo, J. F(2011). PLA2G6 gene mutation in autosomal recessive early-onset parkinsonism in a Chinese cohort. Neurology, 77(1), 75–81. https://doi.org/10.1212/WNL.0b013e318221acd3

Silvestri L, et al. (2017). Carbamoyl phosphate synthetase 1 deficiency: A clinical, biochemical and molecular characterization. Clin Genet, 91(5), 780–785.

Smaili, I., Tesson, C., Regragui, W., Bertrand, H., Rahmani, M., Bouslam, N., … Bouhouche, A. (2021). Gene Panel Sequencing Identifies Novel Pathogenic Mutations in Moroccan Patients with Familial Parkinson Disease. Journal of Molecular Neuroscience, 71(1), 142–152. https://doi.org/10.1007/s12031-020-01635-3

Tomey C. E., Heywood, W. E., Evans, J. R., Lachica, J., Pressey S. N., Foti, S. C., … Palkovits, M. (2022). Mitochondrial dysfunction is a key pathological driver of early stage Parkinson ' s. Acta Neuropathologica Communications, 1–25. https://doi.org/10.1186/s40478-022-01424-6

Trinh, J., & Farrer, M. (2013). Advances in the genetics of Parkinson disease. Nature Reviews. Neurology, 9(8), 445–454. https://doi.org/10.1038/nrneurol.2013.132

Van der Auwera, G. A., Carneiro, M. O., Hartl, C., Poplin, R., del Angel, G., Levy-Moonshine, A., … DePristo, M. A. (2013). From fastQ data to high-confidence variant calls: The genome analysis toolkit best practices pipeline. In Current Protocols in Bioinformatics. https://doi.org/10.1002/0471250953.bi1110s43

Verstraeten, A., Theuns, J., & Broeckhoven, C. Van. (2015). Progress in unraveling the genetic etiology of Parkinson disease in a genomic era. Trends in Genetics, 31(3), 140–149. https://doi.org/10.1016/j.tig.2015.01.004

Volpicelli-Daley L. A., Luk, K. C., & Lee, VM. (2014). Addition of exogenous α-synuclein preformed fibrils to primary neuronal cultures to seed recruitment of endogenous α-synuclein to Lewy body and Lewy neurite-like aggregates. Nature Protocols, Vol. 9, pp. 2135–2146. https://doi.org/10.1038/nprot.2014.143

Von Campenhausen, S., Bornschein, B., Wick, R., Bötzel, K., Sampaio, C., Poewe, W., … Dodel, R. (2005). Prevalence and incidence of Parkinson's disease in Europe. European Neuropsychopharmacology, 15(4), 473–490. https://doi.org/10.1016/j.euroneuro.2005.04.007

W, M. et al. (2018). Urea Levels in Cerebrospinal Fluid and Parkinson's Disease. Frontiers in Aging Neuroscience, 10, 207.

Wüllner, U., Kaut, O., DeBoni, L., Piston, D., & Schmitt, I. (2016). DNA methylation in

Parkinson's disease. Neurochemistry, Journal O F, 139, 108–120. https://doi.org/10.1111/jnc.13646

Yang, C. et al. (2019). Inhibition of carbamoyl phosphate synthase 1 as a therapeutic strategy in a mouse model of Huntington's disease. Experimental Neurology, 312, 58–67.

Yang, Y., Muzny, D. M., Reid, J. G., Bainbridge, M. N., Willis, A., Ward, P. A., … Eng, C. M. (2013). Clinical Whole-Exome Sequencing for the Diagnosis of Mendelian Disorders. New England Journal of Medicine, 369(16), 1502–1511. https://doi.org/10.1056/nejmoa1306555

Zadikoff, C., Rogaeva, E., Djarmati, A., Sato, C., Salehi-Rad, S., St. George-Hyslop, P., … Lang, A. E. (2006). Homozygous and Heterozygous PINK1 mutations: Considerations for diagnosis and care of Parkinson's disease patients. Movement Disorders, 21(6), 875–879. https://doi.org/10.1002/mds.20854

Zis, P., Martinez-Martin, P., Sauerbier, A., Rizos, A., Sharma, J. C., Worth, P. F., … Chaudhuri, K. R. (2015). Non-motor symptoms burden in treated and untreated early Parkinson's disease patients: Argument for non-motor subtypes. European Journal of Neurology, 22(8), 1145–1150. https://doi.org/10.1111/ene.12733

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